List of Published Papers submitted by Mr J. W. Scott Macfie, M.B., B.Sc., as Thesis.

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1. Experiments and Observations upon Glossina palpalis.
2. The Distribution of Glossina in the Ilorin Province of Northern Nigeria.
3. Trypanosomiasis of Domestic Animals in Northern Nigeria.
4. On the Morphology of the Trypanosome (T. nigeriense, n.sp.) from a Case of Sleeping Sickness from Eket, Southern Nigeria.
5. On the Pathogenicity of the Trypanosome (T. nigeriense) from a Case of Sleeping Sickness from Eket, Southern Nigeria.
6. Preliminary Note on the Development of a Human Trypanosome in the Gut of Stomoxys nigra.
7. Sleeping Sickness in the Eket District of Nigeria. (In collaboration with Dr Gallagher).
8. Auto-Erythrophagocytosis as an Aid to the Diagnosis of Trypanosomiasis. (In collaboration with Dr J.E.L. Johnston).
9. Observations on the Action on Trypanosomes of Certain Drugs and of Staphylococcus Pyogenes. (In collaboration with Dr J.E.L. Johnston).
10. A Case of Equine Trypanosomiasis characterised by the occurrence of Posterior Nuclear Forms. (In collaboration with Dr J.E.L. Johnston).
11. A Note on the Occurrence of Spirochaetosis of Fowls in Southern Nigeria. (In collaboration with Dr J.E.L. Johnston).
12. Experiments and Observations on Yellow Fever. (In collaboration with Dr J.E.L. Johnston).
13. Notes on some Blood Parasites collected in Nigeria.
14. A Note on Five Cases of Porocephaliasis in Man from Southern Nigeria. (In collaboration with Dr J.E.L. Johnston).
15. A Note on the Action of Common Salt on the Larvae of Stegomyia fasciata.
16. On a New African Species of Coccidae.
17. Tabanidae attracted by Scale-insects.

Nos.1-7 form a series of investigations on the subject of trypanosomiasis in Nigeria. Beginning with a study of the bionomics of the tsetse fly (No.1), the intermediate host of the trypanosomes /

EXPERIMENTS AND OBSERVATIONS UPON GLOSSINA PALPALIS.

BY

Dr. J. W. SCOTT MACFIE, M.A.

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A note should perhaps be included here on the manner of death of the flies used in the experiments. When an exposure was nearing a fatal termination the flies became less active, and would presently settle on a twig, or on the floor of the jars in which they were contained, in a lethargic condition, being with difficulty persuaded to move, even when touched with the end of a probe. A little later they would fall over on to their backs with their legs pointing up into the air, and their probosces projecting forwards. In this state they might remain for a considerable time. At first, when disturbed, they would be able to struggle to their feet, only to fall over on to their backs again in a few moments. Later they were only able to respond by movements, more or less free, of the legs and proboscis. In this condition, which in the notes of my experiments I have described by the expression "as if dead," they remained until death took place, the movements gradually becoming less perceptible. In exposures to an increased temperature the flies sometimes spread out their wings convulsively just before death. After death the proboscis was generally depressed.

The Effect of a Lowering of the Temperature.

In order to determine the effect of lowering the temperature on *G. palpalis* a simple cold chamber was constructed by filling a large glass jar with ice and salt, or ice and water, in which a smaller glass jar fitted with a thermometer was completely immersed. Into the smaller jar the fly was introduced together with some leafy twigs on which it might alight. In such a chamber it was possible to watch simultaneously the fluctuations of the temperature and the behaviour of the imprisoned fly.

As the minimum temperature recorded at Zungeru in 1910 was 57° F. (13·8° C.), a few experiments were at first carried out at temperatures of this order. It was found, however, that with the exception that the flies became somewhat sluggish, as if numbed, these temperatures had little effect. In one experiment two male flies were exposed to 60–62° F. (15·5°–16·6° C.) for two hours. On removal to the external air at 90° F. (32·2° C.) they appeared to be numbed and were with difficulty persuaded to move. Five minutes later, however, they were quite lively and actively flying about.

The effect of lower temperatures was then examined. The following are the notes of one of the experiments :—

No. 1.—One male and one female *G. palpalis*. Temperature of the external air—24° C. (75·2° F.).

8.5 p.m.	T. 12° C.	Flies introduced into the cold chamber.
8.8	T. 11	Male fell to the ground "as if dead." Female still active.
8.10	T. 10	Female resting on a twig.
8.11	T. 8	Female fell to the ground "as if dead."

Both flies were then removed to a box at the temperature of the external air. The female recovered activity immediately, but the male only after a little time, and was unable to fly until 8.20 p.m.—9 minutes later.

An experiment was then made with a recently captured female *G. palpalis* :—

No. 2.—Female *G. palpalis*. Temperature of the external air—

33° C. (91·4° F.).

12.5 p.m.	T. 10° C.	Fly introduced. She at once settled on the floor of the chamber.
12.15	T. 10	Fly has not moved at all.
12.35	T. 10	Do.
12.40	T. 10	Position unchanged. Stroking wings with hind legs.
12.45	T. 9·5	Do.
12.55	T. 10	Do.
1.5	T. 10	Do. Stroking wings.
1.15	T. 10	Do.
1.20	T. 7	Do.
1.25	T. 7	Do.

The fly was then removed from the cold chamber, and rapidly recovered her activity.

The female *G. palpalis* is therefore able to withstand exposure for an hour and twenty minutes to a temperature ranging from 10°–7° C. (50°–44·5° F.) without harm. This is a degree of cold to which she would never be exposed naturally at Zungeru, where the minimum temperature recorded in 1910 was 13·8° C.

In the case of the male used in the following experiment, twenty minutes exposure to a temperature falling from 16° to 7° C. (60·8° to 44·5° F.) resulted in reducing the fly to an inert condition, from which, however, he recovered rapidly on being removed from the cold chamber.

No. 3.—Male *G. palpalis*.

11.40 a.m.	T. 16° C.	Fly introduced. Settled on a twig.
11.45	T. 10	Fly has not moved.
11.50	T. 9	Do.
11.52	T. 8	Do. Proboscis depressed.
11.55	T. 7·6	Do.
12.0	T. 7	Fly fell off the twig "as if dead."

At once removed to the external air. Recovered activity rapidly.

Temperatures sufficiently low to render the flies inert are not therefore necessarily fatal. In one case a fly was allowed to remain in this condition for five minutes at a temperature ranging from 8°–5·5° C. (46·4°–42° F.), and recovered completely within two minutes after being removed to the temperature of the external air which was at the time 27° C.

That *G. palpalis* can resist relatively low temperatures the following experiment proves. It also indicates the probable limit of low temperature, beyond which recovery does not take place ; as the fly, after having been exposed to a temperature at one time as low as 2·5° C. (36·5° F.), only recovered partially on being removed from the cold chamber, and subsequently died within twelve hours.

No. 4.—Male *G. palpalis.*

8.50 p.m	T. 10° C.	Fly introduced into the cold chamber. He buzzed about for a moment, alighted on the side of the jar, and fell over immediately "as if dead."
8.52	T. 8	—
8.55	T. 4	—
9.0	T. 3	—
9.5	T. 2·5	—
9.10	T. 3	—
9.15	T. 3	—
9.20	T. 3·5	—
9.25	T. 4	Fly removed from the cold chamber having been in an inert condition for 35 minutes. Temperature of the external air—26° C.
9.28	—	Slight movements of the legs.
9.31	—	Abortive attempts at flight.
9.40	—	Still very unsteady on his feet.
10.0	—	Able to fly, but still very uncertain on his feet.

This fly never recovered completely, but died at about 9.30 a.m. on the following morning.

The immediate effect of a lowering of the temperature on *G. palpalis* is to cause a reduction in activity. Should the temperature fall to a point varying from 10° C. to 6·5° C. the fly becomes inert, and falls to the ground as if dead. Partial recovery was found to occur even after half an hour's exposure in this condition to a temperature ranging from 2·5° C. to 4° C. Recovery from less severe exposures was complete, and accompanied by violent buzzing.

In Zungeru, as will be seen on reference to the meteorological return for 1910, given above, such low temperatures as those used in these experiments do not occur. The same applies in the case of the other stations in Northern Nigeria in which *G. palpalis* is found. Practically never does the temperature fall as low as 10° C., a degree of cold which *G. palpalis* has been shown experimentally to withstand for a considerable length of time. No doubt, however, the cold of the harmattan does play a part in the reduction of the number of tsetse-flies during the dry season, by numbing them so that they cannot feed, and by rendering them a more ready prey to their natural enemies.

The Effect of Raising the Temperature.

In considering the effect of a rise in temperature upon *G. palpalis* account must be taken of the highest temperatures to which the fly may naturally be exposed. On reference to the meteorological return for Zungeru, given above, it will be seen that these occur during the months covering the latter part of the dry season and the commencement of the rainy season, and that the maximum temperature recorded in 1910 was 107° F. (or 41·6 C.). High temperatures, therefore, occur both when the degree of humidity of the atmosphere is low, and when it is beginning to rise owing to the onset of the rains ; and account of this had to be taken in planning experiments. It was also recognised at the outset

that a distinction must be made between shade temperatures, and those attained by direct exposure to sunshine. In the following experiments tsetse-flies were exposed to the various temperatures in glass jars fitted with a thermometer. Twigs on which the flies might settle were always present. It may be stated at once that the flies appeared to be much more susceptible to a raising of the temperature than they were found to be to a lowering. In the experiment described above as No. 4, the fly withstood for some time a lowering of the temperature of 23·5° C. (*viz.*, from 26° C. to 2·5° C.), whereas in experiment No. 8, a raising of the temperature only 10° C. (*viz.*, from 34° C., the temperature of the external air at the time of the experiment, to 43°–44° C.) was more than could be endured.

In direct sunlight.

(a.) *In the absence of water.*

In these experiments tsetse-flies were exposed in glass jars to the direct rays of the sun. Small twigs were included in the jars, but no water. The following experiment will indicate the nature of the effects observed.

No. 5.—Male *G. palpalis*. Degree of humidity of the atmosphere—25.

2.4 p.m.	T. 34° C.	Fly exposed to direct sunlight.
2.6	T. 38	Fly active.
2.7	T. 40	Do.
2.8	T. 41	Fly fell to the floor and rolled over "as if dead."
2.13	—	After five minutes in this condition and at this temperature he was removed to a box at the temperature of the external air.

No recovery took place.

Direct sunlight, in the absence of moisture, is therefore rapidly fatal to *G. palpalis*; in this case five minutes exposure at 41° C. having proved fatal.

(b.) *In the presence of water.*

The present experiments only differed from those described above in the fact that water was present in the jars. The following are the notes of one experiment:—

No. 6. Male *G. palpalis*. Water and twigs in the jar. The temperature of the external air was 31·5° C. (88·7° F.).

12.29 p.m.	T. 40° C.	Fly introduced. Active.
12.32	T. 41	Fly active.
12.35	T. 41	Do.
12.37	T. 41	Fly active; looking for shade.
12.40	T. 41	Do. do.
12.43	T. 41	Fly fell over on to his side, but got up again. Less active.
12.44	T. 41	Less active.
12.45	T. 42	Fly fell over again, but got up with difficulty.
12.48	T. 41	Fly crawling about looking for shade, and constantly falling over and getting up again with increasing difficulty.

12.54p.m.	T. 41	Fly fell over and failed to get up again. Slight movements.
12.59	T. 41	After five minutes in this condition the fly was removed to a box at the temperature of the external air. He did not, however, show any signs of life.

In the presence of moisture therefore it would appear that *G. palpalis* is able to withstand exposure to sunlight for longer than in its absence. In this case 25 minutes at 41° C. (105·8° F.) elapsing before complete immobility was induced, as compared with four minutes in the previous experiment (no. 5). A further exposure of five minutes was however fatal.

It was observed that the first effect on the flies of exposure to sunlight was an increased activity. After a few minutes, however, they began to seek shade, and became less active, until finally they were reduced to an inert condition from which recovery, if it took place at all, was slow and often incomplete.

When shaded from direct sunlight.

The jars containing the flies in these experiments were protected from the direct rays of the sun by a covering of thick brown paper. In some experiments water was present in the jars, in others it was absent.

(a.) *In the absence of water.*

The following experiment will indicate the nature of the effects of a raising of the temperature under these conditions :—

No. 7.—Male *G. palpalis* confined in a glass jar, wrapped in thick brown paper so as to shade every part, together with a few twigs, but no water. Degree of humidity of the atmosphere—25.

2.24 p.m.	T. 35° C.	Fly introduced. Active.
2.27	T. 38	Fly active.
2.32	T. 39	Do.
2.35	T. 40	Do.
2.38	T. 41	Fly resting on the floor.
2.43	T. 41	Fly active.
2.50	T. 41·5	Do.
2.55	T. 41	Do.
3.0	T. 40	Do.
3.5	T. 40·5	Fly resting on the floor.
3.10	T. 41	Fly fell over "as if dead." On shaking the jar he got up on his legs again and moved about.
3.15	T. 41	Fly resting on the floor.
3.20	T. 41	Fly fell over "as if dead," and failed to get up again. There were, however, slight movements of the legs.
3.25	T. 41	Slight movements still.
3.30	T. 41	Do. do.
3.35	T. 40	No signs of life.

Fly removed to a box at the temperature of the external air (35° C.), he was, however, dead.

In this experiment a fatal result was only produced after an hour's exposure to a temperature of 40°–41° C. This result should be contrasted with that produced by direct sunlight. In other experiments at this temperature, when the flies were removed from the jars immediately they fell "as if dead," partial recovery took place, but in no case was it complete. It was noticed in one case that the right foreleg remained useless and bent up under the thorax.

(b.) *In the presence of water.*

In the presence of water the effects of a temperature of 40° C. are perhaps a little less severely felt than in its absence, as in one experiment of this nature a male *G. palpalis* was not reduced to a condition of complete immobility by one hour's exposure. On removal to a box at the temperature of the external air he made but a slight recovery, and died within three hours. In another experiment carried out under the same conditions 40 minutes' exposure to T. 40°–41° C. proved fatal, so that the difference if it exists is a small one.

(c.) *Higher temperatures.*

Temperatures higher than those employed in the foregoing experiments (40°–41° C.) are, however, rapidly fatal. The following experiment may be compared with experiment No. 7, which was carried out under similar conditions. In the latter all movements of the fly ceased, and death ensued after 60 minutes' exposure to T. 40°–41° C. (104°–105·8° F.), in the former death resulted after only 22 minutes at T. 43°–44° C. (109·4°–111·2° F.).

No. 8.—Male *G. palpalis* in glass jar shaded from direct sunlight. No water present in the jar, but only a few twigs. Degree of humidity of the atmosphere—27.

2.16 p.m.	T. 44° C.	Fly introduced. Very active.
2.17	—	Fly taking short flights.
2.18	T. 43	Fly on his back; got up again.
2.20	T. 43	Fly on the floor.
2.22	T. 43·2	Fly on his back; struggled up again.
2.24	T. 43·5	Fly on his back, unable to rise.
2.26	T. 43·5	Slight movements.
2.28	T. 43·5	Do.
2.30	T. 43·5	Movements scarcely perceptible.
2.32	T. 44	Do. do.
2.34	T. 43·8	Do. do.
2.36	T. 43·8	Slight movements on shaking the jar.
2.38	T. 43·8	No movements observed.

Fly removed to a box at the temperature of the external air (34° C). Died.

Conclusions.

1. *Glossina palpalis* is able to withstand a lowering of the temperature to 10°–7° C.
2. Direct sunlight is rapidly fatal to *G. palpalis.*

3. When shaded, exposure for one hour to a temperature of 40°–41° C. is fatal to the male *G. palpalis*. Higher temperatures are more rapidly fatal.
4. The presence of water enables *G. palpalis* to withstand for a longer period the action of direct sunlight, and perhaps of a shade temperature of 40°–41° C.

It is probable, therefore, that the diminution in the number of tsetse-flies during the dry season, and their limitation to the bush along the river banks, is due not so much to the low temperatures registered at nights during this season, nor to the high temperatures occurring during the days, as to the dryness of the atmosphere and the burnt up condition of the country.

Notes on the Bionomics of Glossina Palpalis.

The following more or less disconnected observations and experiments were made at Zungeru in Northern Nigeria during December, 1911, and January, 1912. These months cover the height of the dry season when the tsetse-flies (*G. palpalis*) are restricted to a few shady patches of bush along the river banks, probably owing (as indicated by the foregoing experiments) to their intolerance of the dryness of the atmosphere and the burnt up condition of the country. Of the flies brought to me the proportion of males to females was as three to one, but whether this was due to the females being actually less numerous than the males, or to the fact that they were shyer feeders or more cunning at avoiding capture, it is impossible to say. The fact that in some of the temperature experiments the females seemed to be more sensitive than the males to a raising of the temperature may have had something to do with it, as they may have been less eager to fly out into the sunshine to feed and may thus have escaped capture. The converse, namely that the female is apparently less affected by cold, may account for the fact that on one occasion only, on a singularly cool and sunless day, the proportion was reversed, namely three females being taken for every male. The flies were also found to be susceptible to wind, and on the days when the harmattan wind was blowing strongly few or no flies were caught.

Feeding and Digestion.

In considering the processes of feeding and digestion of *G. palpalis* it was found to be essential that the flies should be naturally fed. At first, attempts were made to feed them on such things as the liver of a fowl just killed, or even on freshly shed human blood, but although the flies sometimes succeeded in sucking nourishment from them, they were not able to get a full normal feed, and it was at once recognised that the digestion of such meals was abnormal. In a few instances the flies were fed on dogs, cats and guinea-pigs, but even on such animals the act of feeding did not seem to be quite natural, and often occupied as long as five minutes, whereas on the human body the flies seldom remained longer than one or two minutes, if undisturbed, before flying off fully distended with blood. In the case of the guinea-pigs, the flies showed a curious reluctance to feed even when suffering so severely from starvation that they died a few hours later. The flies were therefore allowed to feed on my own fingers in all the experiments referred to below.

It has been pointed out that movement plays a considerable part in attracting the attention of tsetse-flies, and in his recent report on the Gambia, Dr. J. J. Simpson has given several notable instances of this fact. But whilst it is undoubtedly the case that movement does attract the flies from a distance, I believe that at closer quarters some sense of smell is essential in guiding them. In the glass jars in which I kept my flies, at any rate, as soon as a finger was pressed against the gauze cover the flies came to feed, whereas they ignored any other similar looking body, or even the leg or back of a guinea-pig in the same position. Whether the body applied to the opening was moved about or kept quite still seemed to make no difference.

The Act of Feeding of Glossina Palpalis.

The attitude during feeding is of course well known, the fly standing firmly on all six feet and thrusting its honey-brown proboscis vertically downwards into the skin, whilst the ensheathing palpi project forwards and upwards. As pointed out by Dr. Moiser, the fly sometimes partially withdraws its proboscis and thrusts it in again and again, but once a satisfactory well of blood has been tapped, the abdomen fills rapidly and in from one to two minutes the act of feeding terminates, and the fly, withdrawing its proboscis, moves to one side and presently flies heavily away.

In my experiments I have usually found the bite painless. Sometimes however it was decidedly painful, and I can only account for this by supposing that on these occasions the proboscis entered sufficiently near to one of the sensory spots of the skin to stimulate it. On the other hand there was generally some degree of smarting at the site of puncture after the feed was over, and it is probably this which usually attracts the attention of the victim to the presence of the fly, which having already fed is able to escape on the first indications of uneasiness.

If undisturbed, the fly feeds until the abdomen is relatively greatly distended and of a bright red colour, due to the blood showing through the tense and semi-transparent tissues. The dorsal plates of the abdomen are widely separated, so that the body loses its characteristic dark colour. The appearance is therefore somewhat different from that given in the figure of "A Tsetse fly (*Glossina morsitans*), after feeding" in Mr. Austen's "Handbook of the Tsetse flies" on page 93. To determine the amount of blood actually drawn up, the following experiments were carried out. Two or three tsetse-flies (*G. palpalis*), that from the almost linear dimensions of their abdomens had evidently not been fed for some time, were stupefied with chloroform and in this condition were weighed. They were then allowed to recover consciousness, and when quite restored were fed undisturbed on my fingers. It was noticed that they almost invariably selected the extensor aspect, a precaution that no doubt often saves them from being crushed by a sudden flexion of the finger. They were once again stupefied with chloroform and reweighed. The difference between the two weighings represented the weight of the blood taken up, for sufficient time was not allowed to elapse after the feeding for any exudations to be lost, and in the condition of

stupefaction produced by chloroform the process of digestion appeared to be arrested. The following is an example of one such experiment :—

One male and one female *G. palpalis*, lean and unfed.

12 noon. Stupefied and weighed—·038 gm.
1.0 p.m. Flies completely recovered.
1.30 Allowed to feed on my finger, which they did readily, stupefied with chloroform as soon as they ceased feeding, and weighed —·088 gm.

Therefore the blood taken up by one fly was :—(·088—·038)÷2, or ·025 gm. As the result of a number of such experiments the average weight of blood ingested by the male was found to be ·027 gm. which, reckoning the specific gravity of normal blood as 1·06, corresponds to 2·5 cmm. Larger numbers of flies were not employed in each experiment owing to the difficulty of getting them to feed simultaneously, and the practical impossibility therefore of preventing some of them exuding drops of fluid before being weighed. The advantage in the reduction of the one source of error would therefore have been overbalanced by the introduction of another.

The weight of the flies varies considerably with the length of time that has elapsed since the last meal, but nevertheless there is an appreciable difference between the weights of the unfed males and females. As a rough average the unfed male weighs ·020 gm., and the female, which is somewhat larger, ·028 gm. The same applies to the fed flies—they do not always engorge themselves to the same extent, but the average weight of blood ingested is somewhat greater in the case of the female. On one occasion as much as ·0485 gm. (or 4·5 cmm.) of blood being drawn up by a female, and ·030 gm. by a male. The proportion is perhaps better stated in comparison with the body weight—a male *G. palpalis* is capable of sucking up blood weighing 1·3 times his body weight, and a female 1·6 times her body weight.

With regard to the frequency with which the flies feed it was generally found that once fed they refused to bite again until about 30 hours had elapsed. But here again the intensity of the last meal has to be taken into account, for on one occasion a fly which had fed very fully refused to feed again until 70 hours later. As the flies were all confined in glass jars containing no water this shows that when fully and naturally fed they are able to live unfed much longer than the 30 hours that is sometimes given as the limit of their endurance under such conditions.

Digestion.

Having fed, the tsetse-fly settles quietly to digest. At first the under surface of the distended abdomen is uniformly red in the case of the male, but the female always has a pale opaque spot at the distal end. In from 2 to 5 minutes however a pale bubble appears at the proximal pole situated generally just to the left of the mid line. This bubble shows slight movements of contraction and expansion as though it were influenced by some peristaltic-like action of the bowel, and moves gradually over towards the left side, keeping all the time at the proximal extremity of the abdomen. On dissecting a fly at this stage, the bubble was found to be gaseous, and from the fact that the red tint of the blood lingers along

its edge long after it has faded from the rest of the abdomen, we may surmise that it contains oxygen as a constituent at any rate. Two other processes are meanwhile observed to be going on, namely, the exudation of drops of fluid from the anus, and a darkening of the ingested blood.

It is not until 2 or 3 minutes after the fly has settled down to digest its meal that drops of fluid begin to be extruded from the anus. The first drop is often, though not invariably, of a buff colour and turbid, but the succeeding drops are of clear water-like fluid and are of the size of a pin's head. For about 20 minutes they are exuded at intervals varying from 15 to 75 seconds, the intervals being somewhat longer towards the end of this period. Thereafter no more drops are ejected for several hours, and those that follow are of a thick chocolate brown excrement, which on microscopical examination is found to be composed of little rounded bodies, staining a rich purple with Giemsa's solution, and varying in size from mere dots to discs a third the size of a red blood corpuscle. Around many a more or less disintegrated shell can be made out which is probably the remains of an erythrocyte. Coincident with the extrusion of these drops, which are presumably the fluid constituents of the blood, there is a marked shrinking in the size of the abdomen.

Soon after digestion has begun the appearance of the blood in the distended abdomen of the fly begins to change. Commencing at the distal end and spreading upwards the abdomen loses its bright red colour and gradually darkens, so that in from 20 minutes to half an hour the last tint of red has gone. The last part to darken is a narrow zone around the gaseous bubble, and as has been suggested above, this may be due to the presence of free oxygen in it.

Slight differences were observed in the case of the female *G. palpalis*. When fully distended with blood her abdomen appeared to be deeper, or less dilated laterally, than in the case of the male, and the process of darkening of the ingested blood was considerably more rapid. In one case the last trace of red colour had disappeared from the abdomen in 10 minutes. During the insensibility induced by chloroform the processes of digestion seemed to be arrested, no drops of fluid were exuded until consciousness began to return, and it was often as long as an hour before the last tint of red had left the abdomen. The blood in one fly dissected was found to be perfectly fluid half an hour after it had been ingested.

The Influence of Colour on Glossina Palpalis.

Having previously ascertained that, in the case of the male *G. palpalis* confined in a glass jar without water at a temperature of 40°–41° C. (104°–105·8° F.), whereas exposure to direct sunlight was rapidly fatal, a similar result was only produced after an hour's exposure if the jar was shaded from the direct rays by a covering of thick brown paper, some experiments were carried out to determine whether under similar conditions different colours would produce different results. Male *G. palpalis* flies were therefore exposed to a temperature of 40°–41° C. in glass jars shaded respectively with red, green, blue and yellow paper covers. No water was present in the jars. In the case of the red, yellow and blue covers, after an hour's exposure the flies seemed to be none the worse, and remained very active

throughout the experiments. In the case of green however, after an hour's exposure the fly was showing evident signs of distress, and by continuing the experiment for a further 18 minutes he was killed. The details of this experiment were :—

Male *G. palpalis*, unfed, in jar shaded by an apple green cover.
No water. Temp. of air—32° C.

1.15 p.m.	T. 41° C.	Fly introduced. Active.
1.30	T. 41	Fly active.
1.45	T. 40	Do.
2.0	T. 40·5	Fly quiet on floor.
2.5	T. 41	Do.
2.10	T. 41	Do.
2.15	T. 41	Fly fell over on to his back, but got up again at once when the jar was gently shaken.
2.20	T. 41	Fly quiet on floor.
2.25	T. 41	Hardly able to stand.
2.30	T. 40·8	Fly "as if dead." Slight movements of the proboscis only.
2.33	T. 41	These movements ceased. Proboscis depressed. Fly removed to a box at the temperature of the external air, but he was dead.

Bearing in mind the rapidly fatal result of exposure to direct sunlight, it is rather remarkable that in the semi-darkness of the jars shaded by thick brown paper a temperature of 40°–41° C. should have had a more serious effect on the tsetse-flies than in jars shaded by the various coloured papers. In one typical experiment with a fly in a jar shaded by thick brown paper signs of distress were noticed after 35 minutes, and the fly was dead after a further exposure of 25 minutes.

Zungeru, January, 1912.

THE DISTRIBUTION OF GLOSSINA IN THE ILORIN PROVINCE OF NORTHERN NIGERIA.

BY

DR. J. W. SCOTT MACFIE, M.A.

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THE DISTRIBUTION OF GLOSSINA IN THE ILORIN PROVINCE OF NORTHERN NIGERIA.

By Dr. J. W. Scott Macfie, M.A.,
West African Medical Service.

(Plates I—VII and Map.)

With the rapid development of Northern Nigeria, following the British administration and the completion of the railway from Lagos to Kano, problems hitherto of minor importance are continually coming into prominence. Amongst these the abatement of trypanosomiasis is a matter of medical and entomological interest. As the confidence of the people grows there is an increase of intercommunication, which inevitably involves a danger of the spread of such diseases as sleeping sickness from existing foci. In Northern Nigeria these foci are at present peculiarly isolated, but they will gradually lose their isolation as the efforts made to stimulate the agricultural development of the country meet with greater success and the growing demand for roads, feeders for the railway, and better means of transport, is satisfied. In a densely populated and naturally fertile country like Ilorin the problem of avoiding this danger is particularly insistent, but unfortunately also particularly difficult. In the neighbouring province of Kabba sleeping sickness is said to be endemic, and might readily move westwards with the opening up of the interior ; and in a large part of the province of Ilorin itself tsetse-flies already spread disease amongst the cattle and horses to such an extent that these animals cannot live. Nevertheless, during the dry season, herds of cattle pass, day after day, in an almost continuous stream along the highroads on their way to Lagos from the north. How many die on the journey no one can tell, for the fate of those that sicken is to be butchered by the way, and it is a common experience to come across a carcase hewn up and laid out for sale by the road-side (Pl. I, fig. 1).

The first step towards discovering some remedy for this waste of cattle and horses, and some means of opening up the country safely, would seem to be an adequate study of the distribution of the carriers of the disease, the tsetse-flies ; and it was with this object that, on being appointed to Ilorin in February 1912, I undertook to make a systematic survey of the province.

Physical Features of Ilorin Province.

The Province of Ilorin, which is estimated to contain some 6,300 square miles and supports a population of perhaps half a million of people, forms part of the south-westerly portion of Northern Nigeria. The greater part of the province lies in the area enclosed by the eighth and ninth parallels of latitude and the longitudes 4° and 6° E. On the north it is bounded by the River Niger and the province of Borgu, on the south and west by Southern Nigeria, and on the east by the province of Kabba. It is divided into three sections, the western, the

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eastern or Patigi, and the southern or Offa divisions. These are known respectively as divisions 1, 2 and 3, and each in its turn is sub-divided into a number of districts.

Dr. J. D. Falconer in his "Geology and Geography of Northern Nigeria" thus describes the orographical features of the province :—"The primary watershed of the Niger basin which runs from Kabba to Aiedi and thence westward a few miles south of the Ilorin frontier, is not defined by any range of hills or mountains, but by the crest line of a lofty plain which, like a low, extended arch, slopes gently northwards towards the Niger."* Across this plain six rivers—the Weru, Anwa, Oshin, Oyi, Oro and Kampe—run in an approximately parallel direction northward to flow into the Niger. The ultimate courses of the first two are doubtful and it is probable that before reaching the Niger they join in the manner indicated on the map.

"To the west, Ogga [in Kabba] and Eri are set within a cluster of rocky hills, while to the north there is a comparatively rapid fall to the plains of the Kampi. Between the Kampi and the Niger a steep-sided range of sandstone hills, capped by the pinnacle peaks of Egbom, runs out westward in a succession of rounded ridges two hundred feet above the level of the river, and not until near Share is the river belt of sandstones again bounded by a low escarpment. The Shappa and Orissa Hills form the only prominent range in central Ilorin. The Shappa Hills are of granite, and many of the peaks are gracefully rounded and most picturesque, while the quartzites and gneisses of the Orissa Hills give more rugged and irregular contours. West of the Orissa Hills lies the great plain of Ilorin, thickly populated and well cultivated, in broad undulations trending generally north and south, with scattered inselberge and many kopjes of granite boulders, but with no conspicuous ranges or groups of hills. The Jebba Hills, which extend southward for some distance towards Ilorin, pass westward into an extensive tract of hilly country, high-lying, little known and largely uninhabited, which forms a natural boundary between Ilorin and Borgu."†

The types of scenery are fairly represented by the photographs reproduced in this article to illustrate the haunts of various species of tsetse-flies. Dr. Falconer summarises their characteristics as follows :—"In Kabba, Ilorin, and Borgu, where crystalline rocks of a granitic or gneissic character cover by far the larger part of the surface, the prevailing and most characteristic type of scenery is that of rolling sandy plains with isolated hills or groups of hills rising abruptly above the general level. On the other hand, where sedimentary rocks, such as sandstones and ironstones, cover the surface, as in the river belt of sandstones in Kabba and Ilorin, the characteristic type of scenery is that of steep-sided and flat-topped ranges and plateaux, with detached tabular and conical hills rising from the lower plains."‡

Throughout the greater part of the province the vegetation is open bush, scattered with hardy trees that are able to withstand the annual bush-fires. The country occupying the whole of the central part of Ilorin may be described generally as undulating park-like land, well-farmed and dotted with trees, with

* Geology and Geography of Northern Nigeria, 1911, p. 16.

† J D. Falconer, Geology and Geography of Northern Nigeria, p. 18–19. ‡ *Op. cit.* p. 20–21.

only occasional patches of bush, and thin belts of trees along the banks of the streams. Dense forest-belts occur, however, in the low-lying strips of swamp near the edge of the Niger valley, in the neighbourhood of the Kampe River, in the south-eastern corner of the province, and along the southern boundary as far west as Offa.

On the whole, the province is thickly populated, but there are in the eastern division two districts which are almost entirely uninhabited, the one lying between the rivers Oyi and Kampe, and the other across the Kampe towards Kabba. These districts, which had been depleted of their inhabitants by slave-raiders, are gradually being reoccupied. The north-western portion of the province is also very sparsely populated.

Game is scarce in the more densely populated districts. In the east and the south-east, however, hartebeeste, roan, cob, bushbuck, and duiker occur in fair numbers, and a few waterbuck, reedbuck, and buffalo. Game is also fairly plentiful to the north-west of the River Weru, around Jebba, and in some parts of Shari district.

Climate and Rainfall.

As the distribution of tsetse-flies is intimately associated with the meteorological conditions, a few words must be said about the climate of Ilorin. For this purpose I reproduce below the table of observations for the year 1909 as published in the official Gazette. This year has been selected because it is the latest in which continuous records have been returned for the whole twelve months.

Meteorological Record, Ilorin, 1909.

—	Temperature.				Rainfall.		Wind.
	Shade Maximum.	Shade Minimum.	Range.	Mean.	Amount in inches.	Degree of Humidity.	
January	97	53	44	79.5	.79	65	W.
February... ...	99	71	28	84	1.18	69	W.
March	98	68	30	83.9	5.30	71	S.W.
April	100	67	33	82.3	7.78	76	S.W.
May	98	68	30	81.8	7.12	78	S.W.
June	94	66	28	80.2	8.32	81	S.W.
July	92	67	25	77.3	9.49	81	S.W.
August	92	68	24	77.7	4.92	82	W.
September ...	90	67	23	78.1	14.12	82	S.W.
October	95	65	30	80.3	4.74	82	S.W.
November ...	99	66	33	81.8	.23	70	W.
December ...	102	52	50	78.3	1.19	65	W.
Averages and Totals.	96.3	64.8	31.5	80.4	65.18	75	S.W.

From an examination of this table it will be seen that the climate of Ilorin is humid and equable. The extremes of temperature for the year quoted were 102° F. and 52° F., whilst the mean was 80° F.; the average degree of humidity

was 75, and the rainfall, 65.18 inches, was one of the highest recorded in Northern Nigeria. The province of Ilorin, as might be expected from its geographical position, differs in climate to a marked extent from the more northerly regions. For example, in the greater part of Northern Nigeria the rainfall and temperature curves show only one annual maximum and minimum. In Ilorin, however, the temperature curve tends to show a double crest, and the rainfall, which is conspicuously heavy, rises gradually to a maximum in June, then falls off, only

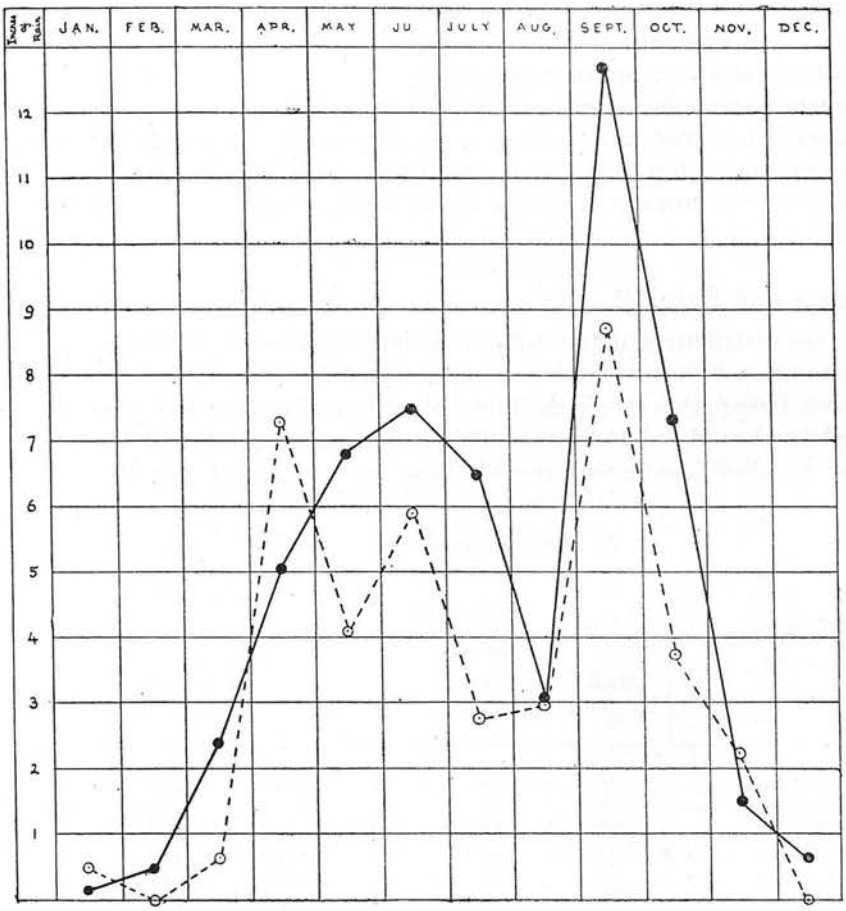

Fig. 1.—Curves showing the rainfall in Ilorin Province; the black line represents the average rainfall for the seven years, 1905 to 1911; the dotted line shows the rainfall for 1912.

to bound up again to its highest point in September. The rainfall is peculiar also inasmuch as normally no month is absolutely rainless, although but little is recorded during December, January and February. The curve reproduced above shows in a graphic manner the average rainfall for the last seven years (1905–1911). The rainfall for the present year (1912) is also shown, as it has been exceptional, a drought occurring from May to August, and it may therefore have had a bearing on the distribution of the tsetse-flies.

Methods of Collecting.

My duties necessitated my presence at Ilorin during the greater part of my tour of service, and it was impossible for me to visit every part of the province personally. I therefore engaged a number of natives, and having trained them to recognise and catch tsetse-flies, sent them out into the different districts as collectors. Of the large number of natives who passed through my hands most were found to be of little use, because, although they were able to catch specimens where they were plentiful, they lacked the perseverance, or some peculiar personal

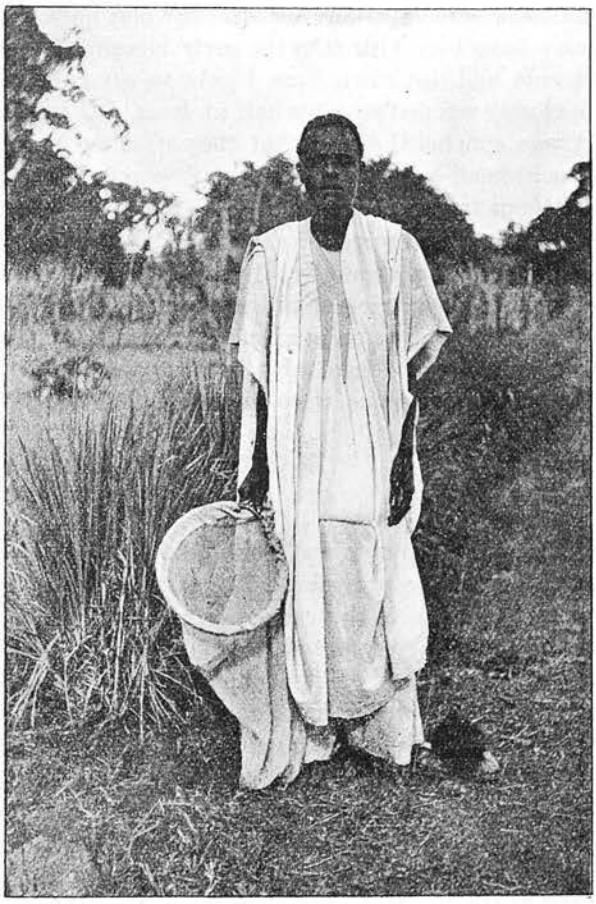

Fig 2.—Umoru, a Fulani of Yola, one of my native collectors.

allurement, necessary to procure them in less likely spots. Eventually, however, I was able to select three or four who showed some aptitude and a degree of discrimination, and by them were made most of the collections on which this report is based. I was also fortunate enough to obtain the co-operation of the Administrative Officers, who interested themselves in the work and not only furnished me with valuable information but also consented to take collectors with them when

on tour in their divisions, thus enabling me to obtain insects even from the most remote corners of the province.

The specimens include representatives from practically every district, excluding only the great uninhabited tracts. Altogether 612 collections were obtained from more than 500 different localities, and so far as is possible these are indicated on the accompanying map by means of the conventional signs suggested by Dr. J. J. Simpson.* As, however, the incompleteness of the geographical survey renders it impossible to mark down all the places accurately, an alphabetical list of them is appended. The list has, of course, a positive value only; and no doubt in many places the flies were overlooked, either because they were present in small numbers, or because the conditions were unfavourable—the day may have been wet or cloudy, or the locality may have been visited in the early morning before the flies were abroad. My own rule, and the instructions I gave to my collectors, was to stop at every stream or closely wooded spot for half an hour. If in this time no tsetse-flies were seen, it was concluded either that they were not present, or that they were present in such small numbers that, for all practical purposes, they were not a menace to cattle or transport animals.

The species of *Glossina* noted were *G. palpalis*, *G. tachinoides*, *G. submorsitans*, and *G. longipalpis*. Speaking generally, it may be said that *G. palpalis* and *G. tachinoides* occurred in all districts of the province. *G. submorsitans*, however, was restricted to the eastern division in a somewhat curious manner.† No flies of this species were taken to the west of a line corresponding roughly with the fifth degree of longitude, and none were found further south than Egbe and Ofa Ora.

The Main Rivers.

Several collections have been obtained from different parts of each of the six main rivers which traverse the province. The actual courses of these streams are largely conjectural; but as, in the case of each river, collections are included which were obtained at widely separated points extending from the vicinity of its source to the neighbourhood of its confluence with the Niger, I do not think that it is an unreasonable assumption to suppose that the intermediate reaches are similarly tsetse-haunted. To substantiate this belief, a short stretch of the Oyun River was examined in greater detail, namely, the six or seven miles which run between the point where the river is crossed by the main road from Ilorin to Balogun and the railway bridge close to the confluence of the Oyun River with the Aza (Pl. I., fig. 2). The accompanying sketch-map shows the tsetse-flies taken at the fourteen points at which the river was examined.

On each of the six rivers *G. palpalis* and *G. tachinoides* occurred, and, in addition, *G. submorsitans* was found to frequent the River Kampe, as well as the northern portions of the Oro and the Oyi.

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* Bull. Ent. Research, ii, Jan. 1912, p. 297.

† In this paper Zumbufu has been included in the eastern division in consideration of its geographical position, although for administrative purposes it is assigned to the western division.

The Western Division.

In the western division of the province the country is thickly populated and well cultivated, and, in consequence, many of the streams are but lightly shaded by trees. Tsetse-flies are correspondingly scarce, and I have often spent an

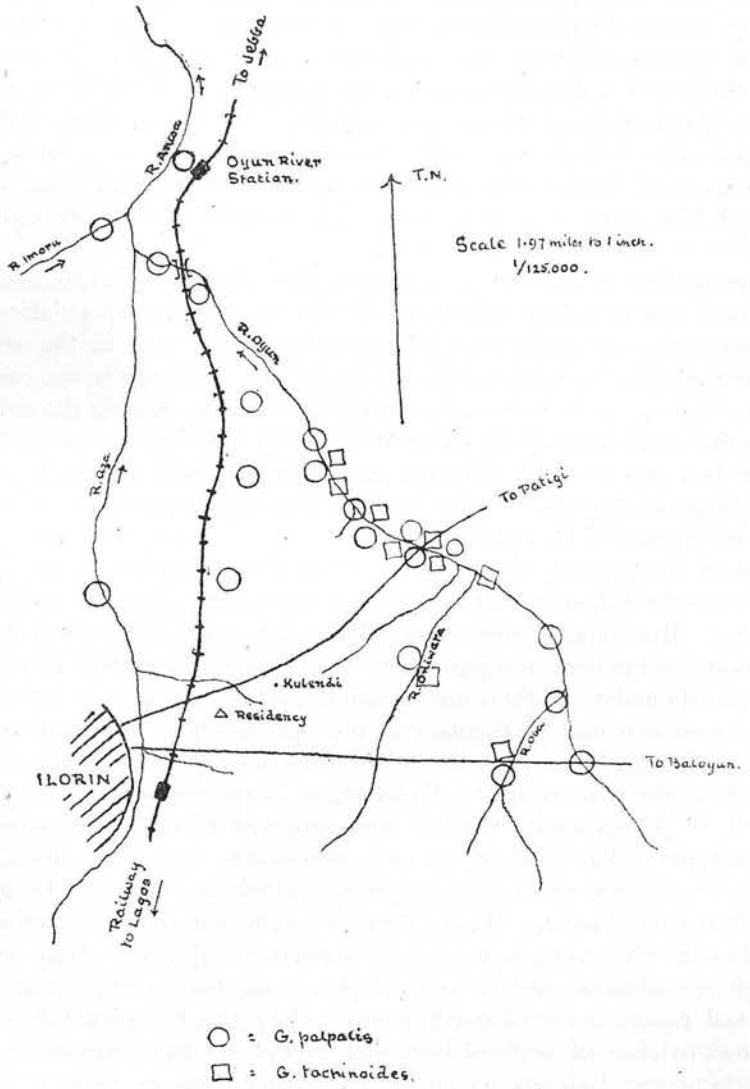


Fig. 3.—Sketch-map of the Oyun River showing the points at which tsetse-flies were taken between the Balogun road and Oyun River railway-station.

hour or more waiting at a likely spot before taking a single specimen. I have, however, never failed to find the flies eventually where there was deep shade and either a stream or pools of water, except in the cases of one or two isolated patches of bush.* In the immediate vicinity of Ilorin town I was able myself to

* *See* p. 13.

investigate the distribution of the tsetse-flies in greater detail, and the results obtained in this district might, I think, be considered to apply to the whole of the great central plain of Ilorin.

G. palpalis and *G. tachinoides* were the only species noted in this division. It was seldom that a distinction could be detected in the haunts of these insects, both being widely distributed over the country, and both, as a rule, occurring beside the streams wherever the conditions were favourable. In some places, however, there was a difference, as, for instance, on the Weru River where it is crossed by the road from Paiye to Ilorin (Pl. II, fig. 1). Here, after taking several specimens of *G. palpalis* in the river-bed, I returned to my horse, tethered, as I hoped, out of harm's way in an open space some distance from the river, only to find him tormented by a dozen *G. tachinoides* which were biting at his fetlocks.

Nowhere in this division were the tsetse-flies sufficiently numerous to be a scourge ; and this fact alone, whether it be due to the denser population and the greater proportion of agricultural land or to some difference in the vegetation, constitutes a remarkable contrast to the conditions obtaining in the eastern and southern divisions. It is, moreover, noteworthy that in crossing the uninhabited district to the north-west of the River Weru, I did not discover tsetse-flies until the River Iwa was reached, although the vegetation was of the type that is generally frequented by *G. submorsitans*, and the district was reputed to be well stocked with game (Pl. II, fig. 2).

In spite of the general occurrence of tsetse-flies, numerous herds of Fulani cattle are seen throughout the greater part of the western division, and appear to thrive there. Moreover, it would be a matter of no great difficulty to render the roads almost everywhere comparatively free from tsetse-flies, owing to the relatively small numbers of these insects and the strict localisation of their haunts. This step is an essential preliminary to the opening-up of the country and the introduction of animal transport, as the following incident proves. In June 1912, I undertook, at the request of the Resident, to locate the tsetse-flies on the road from Ilorin to Agugi, along which it was proposed to try an experiment with donkey transport. The road, which runs almost due east from Ilorin, is about thirty miles in length, and traverses country which is overgrown by grass, and dotted with scattered trees. Where there is a sufficient depth of soil above the rocks, and in the river valleys, numerous farms occur, planted mainly with yams, maize, and ground-nuts ; and at several places, as, for instance, around Iliapa, Balogun, and Agugi, herds of cattle graze. Only at a few points does the road pass through patches of orchard land, and, except on the river-banks, there is between Ilorin and Lalenka no cover sufficiently deep to form a haunt for tsetse-flies. The road crosses three rivers—the Oyun, the Oshin, and the Oyi—and a number of lesser streams, tributaries of these. The banks of all the streams are narrowly fringed with trees and undergrowth which in most cases harbour tsetses, although the depth of the fringe is never more than a few yards. Tsetses were taken on the banks of each of the three main rivers near the points where the road crosses them ; they were also taken on one or more of the tributaries of each. The species was in every case *G. palpalis* ; but at one river—the Oyun—*G. tachinoides* was found in addition (Pl. I, fig. 2). Under these circumstances

it seemed reasonable to assume that the presence of tsetse-flies on any of the streams on the Agugi road is determined by the suitability of the conditions, the most important of which appears to be the presence of deep shade ; and I came to the conclusion that, provided the crossings were properly cleared, the road might safely be used for animal transport. Unfortunately, it was necessary to send out the first drove before the clearing could be effected, and ten healthy donkeys left Ilorin for Agugi on 4th August. On 14th September only three survived, all suffering from trypanosomiasis ; and the last of these died on 1st November, within three months of the beginning of the experiment.

The Eastern Division.

In the eastern division of Ilorin province there are two great uninhabited districts lying on either side of the Kampe River, and a third smaller uninhabited tract just south of Zumbufu. Although it was not possible to examine this division with the same thoroughness as the western, yet the 188 collections obtained are representative of all the inhabited regions, and are probably typical of the whole division.

The tsetse-flies collected were of the following species : *G. palpalis*, *G. tachinoides*, *G. submorsitans*, and *G. longipalpis*. On referring to the map it will be seen that the first three species are distributed over practically the whole district, the only important exception being the country lying to the west of Oke Odde, where only *G. palpalis* and *G. tachinoides* were found. *G. longipalpis* was taken in only a few localities, and as they are widely separated, it is possible that this species has been overlooked in the intervening areas.

The vegetation of this division is generally closer than in the western division, the country is less widely farmed, and in places there are actual belts of forest. This, no doubt, accounts in part for the general distribution of *G. submorsitans*, a species which is absent from the western division. Many of the collections which contained specimens of *G. submorsitans* were, however, made actually on the banks of streams, and there seems to be no doubt that, during the dry season at any rate, this species of tsetse is to be found in considerable numbers along the rivers. On the banks of the Noako River, for example, *G. submorsitans* was abundant during the dry season ; but later in the year, in October, towards the end of the rains, they were conspicuously less numerous. At several places where tsetses of the *palpalis* group only were found during the dry season, *G. submorsitans* was present in addition during the rainy season, a fact that considered in conjunction with the preceding observation, suggests that this species takes refuge in the dry season near the rivers. It would, therefore, be interesting to ascertain whether, by clearing the banks of this stream so as to deprive them of this refuge, the subsequent dissemination of *G. submorsitans* in the neighbouring country might not be checked, as it was found to be in the case of *G. palpalis*, by the clearing of the belt at Offa.* In the dry season, moreover, the vicinity of the Noako River is the haunt of big game. As might be expected from the nature of the country, tsetse-flies are much more common

* *See* pp. 12–13.

in the eastern than in the western division ; in some places, as, for instance, on the River Noako, they are a serious inconvenience.

Along the northern border of this division, from Patigi to Lafiagi and on to Zumbufu, runs the main caravan highway through the province, and during the dry season a constant succession of droves of cattle passes along this road on the way to the Coast. The herdsmen of the north have not yet adopted the facilities offered by the railway for transport, and still frankly prefer the slow but familiar method of driving their beasts hundreds of miles by road. Many of the herds come from Kano and even more distant regions, and the toll that they pay by the wayside must be immense. As they pass through Ilorin province they must trek, between Patigi and Zumbufu, through country infested with *G. submorsitans*, in which the local herdsmen have found it impossible to rear cattle ; * that many of their beasts sicken in consequence and have to be slaughtered is only too certain. In Ilorin town, for instance, which is reached some days after leaving the eastern division, large numbers of sick animals are butchered, and, on making an examination of their blood, I found that the majority were suffering from trypanosomiasis. In most cases the species of trypanosome was *T. vivax*. It is probable, therefore, that the animals had been infected prior to their entry into Ilorin province, for, according to Sir David Bruce and his collaborators on the Sleeping Sickness Commission in Uganda,† the average incubation-period of *T. vivax* in cattle is eighteen days, and the average duration of the disease 89 days. As the cattle slaughtered in Ilorin were often in an advanced stage of trypanosomiasis, these figures would necessitate dating back the infection to a time long before they crossed the Niger. The Nigerian strain may not, however, be identical with that of Uganda, and may possibly have a shorter period of incubation. In horses, at any rate, the onset appears to be rapid, for I have seen a pony in an advanced stage of the disease 14 days after the earliest possible date of infection, and another which showed undoubted symptoms only four days after entering tsetse country.

Unlike the western division, which is well stocked with cattle, the eastern division possesses no Fulani herds, excepting in the extreme westerly portion of the Oke Odde district, in an area near the River Oyi, bounded to the east by a line running from Famali to Oke Odde, and southwards as far as Ora. In the Annual Medical Report on Northern Nigeria for the year ending 31st December 1910, Dr. M. Cameron Blair, Senior Sanitary Officer, wrote : "In Northern Nigeria we fortunately possess, apart altogether from scientific observation, a very good rough-and-ready means of determining the distribution of tsetse-flies at any given part of the year. The Fulani, over the greater part of the Protectorate . . . . possess most of the cattle in the country . . . . For centuries these people have been in the habit of wandering all over the country in pursuit of pasture for their cattle and other live-stock. Apart altogether from the question of the absolute presence or absence of water, these people avoid certain parts of the country at certain seasons, and shun other

* An enterprising herdsman recently attempted to settle near Lafiagi. In September, shortly before I left Ilorin, I heard that his cattle were dying off, thus once more proving the unsuitability of this district for Fulani herds.

† Reports of the Sleeping Sickness Commission of the Royal Society, no. xi.

regions at all times. Now many of those areas, thus permanently or intermittently avoided, appear, to the cursory observer, ideal cattle countries; but their long experience has taught those people that, if they do not act as described, their cattle will die. They believe that in the regions avoided the water is either permanently or intermittently poisonous. . . . Investigation invariably shows that the water itself is not poisonous, but is haunted by tsetse-flies." The eastern division of Ilorin province is one of the areas shunned *at all times*, although the country appears to afford excellent grazing grounds; and this is doubtless due, as Dr. Blair points out, to the presence of tsetses. But I have already stated that tsetses haunt practically every stream in the western division, and that nevertheless cattle are abundant. There is, however, one great difference between the two districts: whereas *G. submorsitans* occurs all over the eastern division, it is apparently completely absent from the western; and this being so, I think the assumption is reasonable that it is this species that is inimical to live-stock. If further proof be necessary, it is furnished by the fact that the one district in the eastern division in which cattle are kept is the only one in which *G. submorsitans* has not been found.

The Southern Division.

The southern or Offa division of the province has been less thoroughly surveyed for tsetse-flies than either of the other two, and the tour on which most of the entries on the map are based was made in September, a very wet month, when circumstances were unfavourable for collecting. Nevertheless the 79 collections were obtained from localities fairly evenly distributed over the whole division, and are probably representative. The only tsetses taken were *G. palpalis* and *G. tachinoides*. *G. palpalis* appears to be distributed over the whole division, and *G. tachinoides*, although caught at only a few places, may be more widely distributed, at any rate in the western districts, than is indicated on the map. The vegetation in this division, however, is less open than in the western division, and resembles the typical habitat of *G. palpalis* more closely than that of *G. tachinoides*. It has been pointed out by Roubaud* that, in West Africa, *G. palpalis* is gradually replaced by *G. tachinoides* as an advance is made inland beyond the forest belts. In this respect the province of Ilorin would seem to cover the intermediate zone, *G. palpalis* predominating in the southern division, but occurring in approximately equal proportions with *G. tachinoides* a little further north, in the western and eastern divisions.

The most remarkable feature of the survey is the apparent absence of *G. submorsitans*, a species which occurs in large numbers in the southern part of the eastern division. It is possible, of course, that the fly may have been overlooked; but it is improbable that no single specimen should have been taken if the species is at all common. From the fact that Fulani cattle are not taken to graze in the southern and eastern parts of the division it was anticipated that *G. submorsitans* would have been found; and, if it is really absent, some other explanation than the presence of this fly must be given to account for the fact that the greater part of the region is shunned by herdsmen.

* Comptes Rendus de l'Académie des Sciences, 2nd Oct. 1911.

In many parts there is thick bush, and the tsetse-flies are very numerous, either of which circumstances might deter the Fulani from leading their herds here to graze; moreover, the more easterly districts would be reached most naturally from the north, when a wide stretch of country infested by *G. submorsitans* would have to be crossed. There is no doubt, however, about the fact that infection with *T. brucei* can be incurred in this division, and the experience of successive Residents has been that the ponies they use on tour in the eastern districts almost invariably die. Game is fairly plentiful in certain parts of the division, as it is in the eastern or Patigi division, whereas it is scarce in the western division; and it is possible, therefore, that trypanosomiasis may be maintained, in this instance, by infected game, and transmitted from them to any horses or cattle that venture into the neighbourhood.

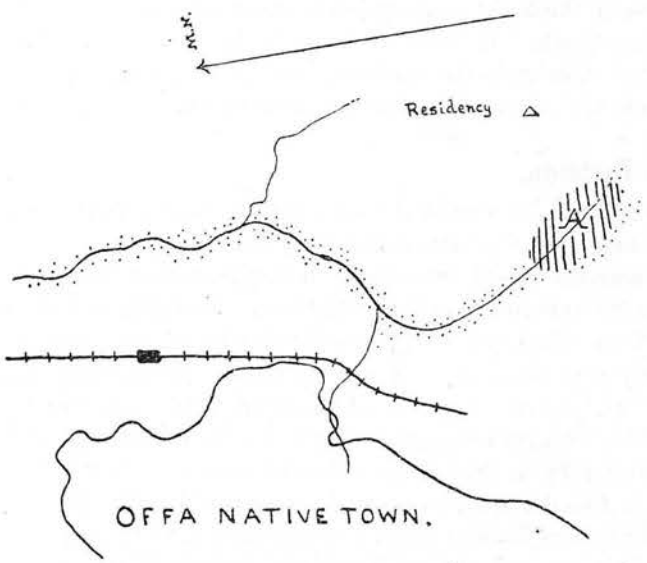


Fig. 4.—Plan of Offa, (A) being the tsetse-haunt.

At Offa itself, which is not only a large native town, but also an important station of the railway, only *G. palpalis* was found. On the western side of the line lies the native town, and on the eastern the residential quarters for Europeans; on the latter side the ground rises gently and at the foot of the slope a small stream runs northwards in a direction parallel to the railway. The sloping ground is divided in two places by narrow valleys, on the ridge between which the Residency stands, and but a little way to the south lies an isolated patch of marshy ground from which originates a small stream. When I visited Offa, in April 1912, this patch of marshy ground (marked A on the plan) was covered with trees and undergrowth, and was found to be the haunt of abundant tsetse-flies (Pl. III, fig. 1).

The rest of the station was sparsely wooded and apparently free from these insects; but as I was assured that during the rains they were sometimes seen even in the bungalows, I concluded that during the dry season the swamp formed

a retreat for the flies, whence they spread over the surrounding country during the rains. I therefore recommended that all the undergrowth should be cleared away, and that the marsh itself should be drained by digging a ditch through the middle. This was done, apparently with good results, for although the haunt was thoroughly searched in July, August and September—that is during the height of the rains—no tsetse-flies could be found; and the European residents informed me that they had not observed any in their offices or bungalows.

As already stated there are in the western division some isolated patches of bush, skirting the smaller streams or surrounding pools, in which I was unable to find tsetse-flies although the local conditions appeared to be suitable. This fact suggests that, in the event of a patch of bush becoming isolated by cultivation or otherwise, it remains for some time a tsetse-haunt, and as in the case of the swamp at Offa, serves as a retreat for the flies in the dry season; but that eventually its tsetses die out, a process that may be accelerated by clearing away all undergrowth.

The Small Race of G. palpalis found in Ilorin.

By far the most common tsetse-fly in Ilorin is one which differs markedly in external characters from any of the recognised species, but which has been identified as *G. palpalis* by both Professor Newstead and Mr. Austen. It is a small fly, with abdominal markings practically identical with those of *G. tachinoides*, with the one exception that the buff colour of the paler areas is replaced by a very characteristic grey-blue tint. Specimens were sent to the Entomological Research Committee, and were very kindly examined by Mr. Marshall, who wrote saying that he could not regard them as being specifically distinct from *G. palpalis* because the genitalia of the males* were practically inseparable. In another letter he wrote that they constituted the principal difficulty in dealing with my collections, for he had found discoloured specimens of *G. tachinoides* extremely hard to distinguish from this very small race of *G. palpalis*.

* In a fresh preparation the details of the genitalia of the male differ considerably from those seen after treatment with caustic potash. Maceration, as might be expected, destroys all the finer structures, and distorts the natural arrangements of the organs. This is conspicuous in the case of the inferior clasper. In a macerated specimen the terminal portion of the inferior clasper is extended into a leg-like process, ending in a foot-like extremity which overlaps its fellow of the opposite side in the middle line. In a fresh specimen, on the contrary, the leg-like process of the inferior clasper is seen to be bent upwards into an S-shaped structure. The first bend occurs at the proximal end of the process in an upward and outward direction, the second in the middle of the foot-like extremity in such a way as to curve the toe upwards and backwards over that part of the extension which, to preserve the analogy, must be called the ankle. Looking at the hypopygium from above, therefore, in a specimen from which the point of the superior clasper has been broken off on one side, a clear outline of the broad basal portion only of the inferior clasper can be seen at the lowest focus. On raising the focus a little the ankle of the leg-like process comes into view, with the sole of the heel and the folded border of the toe forming a line parallel to, but behind, the lower incurving border of the broad basal part of the clasper. At the highest focus only the toe is seen clearly, its tip pointing directly backwards. In a side view the extension of the inferior clasper resembles a snake poised with its head drawn back ready to strike.

The plate (Pl. VII) indicates how unlike these tsetse-flies are to the typical *G. palpalis*, but in view of the structure of the male genitalia, it is, I suppose, only possible to relegate them to that species.

Tsetse-flies of this type were collected from every part of the province and were often found associated with typical *G. palpalis* and *G. tachinoides*. At only a few places were typical *G. palpalis* found alone. A larva deposited by one of these flies measured 4 mm., by 1.5 mm., by 1.5 mm.

Mites found on Tsetse-Flies.

On a few specimens of both *G. palpalis* and *G. tachinoides*, taken during the months of May, June, and August, minute red mites were found attached to the abdomen, thorax, or legs. They were easily detached by the point of a brush, and in one instance the mite voluntarily left the tsetse-fly and was found crawling over the floor of a store-box. They were of two distinct types, and although at the same seasons other mites were common on such insects as mosquitos, *Chrysops*, *Stomoxys*, etc., the forms found on the tsetse-flies were not observed on any other insects. Specimens of these mites were submitted to Mr. S. Hirst, of the British Museum, who very kindly examined them, and expressed the opinion that they were larval forms of TROMBIDIIDAE, and probably belonged to the genus *Trombidium* in its wide sense.

Fulani Cattle.

The *jangali*, or cattle-tax, levied at the rate of 1*s.* 6*d.* per head on all the Fulani cattle in the province, furnishes interesting statistics as to the distribution of the herds. The table given below shows in the first column the sum collected in each district for the year 1911, in the second the approximate area of the districts, and in the third the number of head of cattle per square mile. The figures, which are, of course, only approximate, indicate with accuracy the districts in which the experience of generations has taught the natives they may safely graze their herds.

Distribution of Fulani Cattle in Ilorin Province.[*]

District.	Amount of jangali in pounds (1911).	Approximate area in square miles.	Head of cattle (to the nearest whole number) per square mile.
WESTERN DIVISION.			
Adio	63	40	21
Agodi	34	120	4
Ajidungari	102	100	14
Akambe	59	180	4
Ariore	78	110	9
Awodi	58	100	8
Igporin	193	150	18
Ilorin Town	35	100	5

[*] The *jangali* tax is levied on all Fulani cattle at the rate of 1*s.* 6*d.* a head. No tax is collected on the dwarf cattle found in Egba, Eri, Ofa Ora, Oro, Osi, Idofin and Awtun.

District.	Amount of jangali in pounds (1911).	Approximate area in square miles.	Head of cattle (to the nearest whole number) per square mile.
WESTERN DIVISION—*continued.*			
Kulendi	34	18	25
Lanwa	214	220	13
Malete	85	180	6
Okemi	36	40	12
Oke Moro...	229	600	5
Oke Oyi	74	120	8
Oloru	75	80	12
Shao	24	30	11
Shari	50	330	2
Shonga—no *jangali.*			
SOUTHERN DIVISION.			
Afon	193	150	18
Ajassepo	196	300	9
Igbaja	240	240	13
Isanlu	76	180	6
Awtun, Offa, Omu, Oke Awra, and Osi—no *jangali.*			
EASTERN DIVISION.			
Oke Odde...	40	800	1
Egbe, Ejiba, Eri, Lade, Lafiagi, Ndacheko, Ndeji, Pada, and Patigi—no *jangali.*			

A closer examination into the distribution of the cattle leads to a still further restriction of the area in which they occur. In Shari district, for instance, they are confined to Babanloma, and the neighbouring town of Ajikaji; and in Lanwa district there are no cattle north of Bode Sadu, although they are plentifully scattered throughout the southern portion of the district. Similarly, in the Patigi division the presence of Fulani cattle is limited to the extreme westerly portion of Oke Odde district, to an area bounded on the west by the River Oyi, and on the east by a line drawn from Famali to Oke Odde, and thence to Ora. There are no cattle south of Ora. In Afon, too, the majority of the cattle are found north of Ojuko, and the three or four Fulani encampments in Oke Awra district are all on the Ajassepo boundary. A glance at the accompanying map, on which the districts where Fulani cattle occur are shaded, will show that the cattle are restricted to the western half of the province, no *jangali* being collected in the eastern or Patigi division, and none in the districts along the southern and south-eastern borders. The province of Ilorin may therefore be considered to be divided roughly into two portions by the fifth degree of longitude. Experience has taught the herdsmen that to the west of this line they may safely lead their cattle to graze, but that to the east only disease and death await them.

On comparing the map which represents the distribution of Fulani cattle with the other map showing the distribution of tsetse-flies, it will be observed that the districts in which cattle are absent are, with the exception of those in the south-western corner, almost co-extensive with the districts in which *G. submorsitans* has been found. The correspondence is so close that it suggests that the presence

of this insect is the reason why cattle cannot live in these districts. As a rule the native herdsman is either unable to give any reason at all for the death of his cattle, or attributes it generally to "bad water"; the one exception of which I have heard in Ilorin was in the case of a Fulani at Lafiagi, who told Mr. Budgen, the Resident in charge of the Patigi division, that there was a fly which killed cattle in that district. On being shown a collection of tsetse-flies he singled out one, which Mr. Budgen believes to have been a specimen of *G. submorsitans*, as the sort to which he referred. That Fulani cattle live and breed, apparently in perfect health, in districts in which *G. palpalis* and *G. tachinoides* are widely distributed, is undoubtedly true; indeed it is an everyday occurrence to see herds of cattle grazing beside the streams at the very spots where these tsetses have been found to be most numerous.

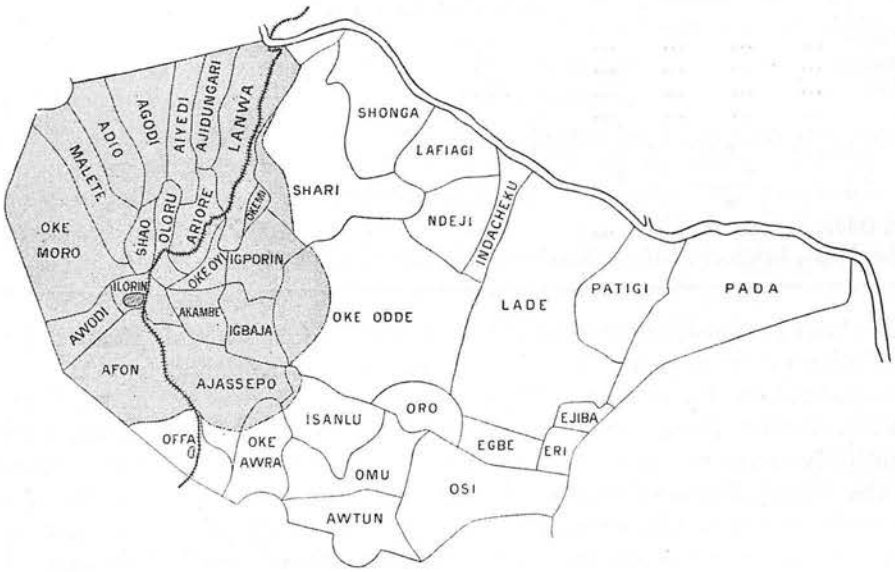


Fig. 5.—Sketch-map showing the distribution of the herds of Fulani cattle in Ilorin province.

Elsewhere* I have pointed out that in Northern Nigeria the trypanosomiasis due to *T. brucei* is much more fatal to horses than that due to *T. vivax*. If the same statement applies to cattle, this fact may partly explain the restricted distribution of the herds in Ilorin province, for *T. brucei* seems to be more common in the east and south-east than it is in the west. In Uganda, moreover, Sir David Bruce and his collaborators† concluded that the carrier of *T. vivax* was probably *G. palpalis*, and it is well known that *G. morsitans* can transmit *T. brucei*.

Dwarf Cattle.

In addition to the Fulani cattle, a few dwarf cattle are met with in the southern and south-eastern districts. A herd of these curious animals is seen amid natural

* Annals of Tropical Medicine and Parasitology, vii., no. 1, 1913.

† Reports of the Sleeping Sickness Commission of the Royal Society, no. xi.

surroundings at Eri in Plate IV. They are found in the neighbourhood of villages and towns in districts in which Fulani cattle cannot exist, and they have therefore been credited with a natural immunity to trypanosomiasis.* At Ilorin, however, I was able to prove that, in one case at any rate, infection with *T. brucei* terminated fatally, although in two cases recovery from infection with *T. vivax* was observed to occur.† It is not likely therefore that these cattle would be of any use as transport animals even if they were to prove physically capable of the work. There is some reason to suppose that these cattle, even when living in country haunted by *G. submorsitans*, may escape the attacks of these insects, as collections of biting flies made at the spots where they were actually grazing did not include this species, although specimens of both *G. palpalis* and *G. tachinoides* were taken.‡

Trypanosomiasis.

It is a fortunate circumstance that sleeping sickness does not appear to occur in an epidemic form in Northern Nigeria, in spite of the fact that tsetse-flies are very generally distributed over the Protectorate. Sporadic cases have been reported from a number of localities, and certain districts, as, for example, the province of Kabba, appear to be endemic centres of the disease. In Ilorin province, human trypanosomiasis is either very rare or altogether absent. The missionaries both at Shonga and Patigi, however, have reported cases of a fatal disease characterised by enlargement of the cervical glands and lethargy which may have been sleeping sickness. The presence of the trypanosome has not been demonstrated, and until this has been done it would be rash to make a definite diagnosis. Elsewhere in the province the disease is unknown, and no cases of even a suspicious nature came under my notice during the nine and a half months I was in Ilorin. This may be due to the fact that the native population is relatively immune to the disease, or to the absence of the species of trypanosome (*T. gambiense* and *T. rhodesiense*) which are pathogenic to man. The comparative scarcity of game, which has been proved by Kinghorn and Yorke to be the reservoir of the sleeping sickness parasite in Rhodesia, should also be remembered in considering this subject. There is indeed some reason to believe that the native population is less susceptible to trypanosomiasis in West Africa than it is in other parts of the continent. Such an opinion has been expressed with regard to sleeping sickness on the Gold Coast, and the few cases of the disease that I have met with in Northern Nigeria have been isolated instances occurring in districts in which the whole population was exposed to the attacks of innumerable tsetse-flies. In the opening up of the country it is, however, of the greatest importance to guard against the possible spread of sleeping sickness; for every development is accompanied by the introduction of individuals, both Europeans and natives, who are certainly not immune. As has already been pointed out, Ilorin occupies a somewhat precarious position from its proximity to Kabba. It

* "A third report on experimental work on Animal Trypanosomiasis," by H. Andrew Foy, D.P.H., in the Journal of Tropical Medicine & Hygiene, 16th Oct. 1911.

† Annals of Tropical Medicine and Parasitology, 1913, vii., no. 1.

‡ *Ibid.*

is moreover impossible to assert that the trypanosomes pathogenic to man do not occur in the province. A polymorphic trypanosome which is morphologically indistinguishable from *T. gambiense* is unfortunately very common in domestic animals, and in a few cases parasites with posterior nuclei have been found which resemble *T. rhodesiense*. But even if these parasites should prove to be *T. brucei*, the danger of man becoming infected from domestic animals is not altogether eliminated, for it has been suggested by Sir David Bruce that *T. brucei* and *T. rhodesiense* are one and the same trypanosome.*

But apart altogether from the danger of sleeping sickness, the question of the abatement of trypanosomiasis of domestic animals merits consideration. This disease is exceedingly common. Trypanosomes presenting the morphological characters of *T. brucei*,† *T. vivax*, *T. nanum* or *pecorum*, and *T. theileri* have been found in Ilorin in the blood of domestic animals; *T. brucei* in the horse, donkey, Fulani cattle, dwarf cattle, and sheep; *T. vivax* in the horse, donkey, Fulani cattle, dwarf cattle, sheep, goat, and dog; *T. nanum* or *pecorum* in the horse, Fulani cattle, and sheep; and *T. theileri* in the dwarf cattle.‡ As the insects that transmit the diseases to man and animals are the same, any measures adopted with a view to diminishing the spread of the latter will apply also to the former. The province of Ilorin, and indeed a large part of the rest of Northern Nigeria, suffers heavily from losses due to trypanosomiasis of horses, cattle, and other domestic animals; and the wealth of the country is curtailed to an even greater extent by the restriction of the districts in which stock can be reared. But for tsetse-borne diseases, cattle might be raised over a very much wider area of the province than is at present the case, and the numbers of sheep and other domestic animals might be greatly increased. It would moreover be possible to introduce some form of transport by means of animals, thus opening up the country more effectually, and liberating a large number of native hands for agricultural employments. In a foregoing section of this paper I have remarked that the eastern division of Ilorin province is one of the regions shunned by Fulani herdsmen, and I have suggested that of the tsetse-flies occurring in this part of the province it is *G. submorsitans* that is particularly inimical to cattle. It may be assumed at once that it is impossible to clear

* There can be no doubt that under certain conditions trypanosomes of the types generally found in animals may infect man. This fact is illustrated by the case recently published by Martin and Darrè ("Un cas de trypanosomiase humaine contractée au laboratoire." Par Louis Martin et Henri Darrè. Bulletin de la Société de Pathologie Exotique, Tome V., No. 10, 1912) in which the trypanosome was reported to have the following reactions: "Pour le virus laboratoire, très pathogène pour la souris, et manifestement du type nagana-surra, les réactions d'immunité passive croisée (pouvoir protecteur du sérum d'animaux, cobayes, chèvres, infectés) l'éloignent des *Tr. brucei* et *togolense* et le rapprochent du *Tr. evansi*."

† Considerable confusion seems to have arisen as to the type of trypanosome to which the name *T. brucei* properly applies. It should be pointed out therefore that in this paper the name *T. brucei* has been used when referring to a polymorphic parasite similar to that described and figured by Sir David Bruce and his collaborators in the Reports of the Sleeping Sickness Commission of the Royal Society, no. xi. This trypanosome is probably that for which Prof. Stephens and Dr. Blacklock have recently proposed the name *T. ugandae*.

‡ See "Trypanosomiasis of Domestic Animals in Northern Nigeria," Annals of Tropical Medicine and Parasitology, vii, no. 1, 1913.

the whole of this country and to exterminate the tsetse-flies. What would be a very difficult and costly undertaking in a district harbouring only *G. palpalis* is an impossible one where *G. submorsitans* is concerned; and the clearing, even if once carried out, would have to be maintained year after year, or close undergrowth, the type of vegetation that is most favourable to these insects, would soon spring up. What then could be done? From the study of the tsetses in the western division of Ilorin it is evident that it is quite feasible to raise stock in districts in which these flies (*G. palpalis* and *G. tachinoides*) are widely distributed, provided that the insects are not too numerous, and are localised in definite haunts. No doubt under more favourable conditions many more herds of cattle could be maintained in this area, but the fact remains that the Fulani herdsmen already regard the country as affording good grazing-grounds, in spite of the fact that practically every stream is a tsetse-haunt. It is not therefore necessary to despair of finding grazing-grounds in some parts of the eastern and southern divisions. The country appears to be admirably adapted to such uses in many places, if only some means could be devised to protect the cattle from the attacks of tsetse-flies. The observations on the dwarf cattle recorded above suggest that this might be accomplished, and the well known fact that tsetses cannot survive exposure to direct sunshine for more than a few minutes indicates the nature of the measures that might be adopted. It would be necessary to maintain a wide area cleared of all undergrowth round the villages and towns, and to direct a number of similarly cleared roads and tracks from this centre towards the farm lands and grazing-grounds. The banks of the streams would also require to be cleared for some distance on either side of the local water supply, and at the points to which the cattle were led to drink. It might in some cases be possible to institute village grazing-grounds, suitably enclosed, and with access to a cleared reach of a stream, in which the smaller domestic animals might be confined. It would of course be necessary to change the site of these plots from time to time, but the land thus fertilised would be valuable for agricultural purposes, and by this means additions would be made automatically to the cleared areas around the villages.

Throughout the province the main roads should be cleared, and the banks of the stream for some distance on either side of the crossings. The natives should be encouraged to farm the road-sides, as cultivation has proved to be the most effective way of exterminating tsetse-flies. Not only would this protect the local cattle when moving from pasture to pasture, but it would tend to diminish the spread of infections introduced by herds passing through the province on their way to the coast. These herds trekking down from the north are a serious source of infection. For the most part they enter Ilorin by crossing the Niger at four points—Jebba, Ogudu, Likpata and Patigi—and the most natural course would be to examine the herds on their arrival at these places, and to prevent any animals suffering from trypanosomiasis from proceeding on their journey, either slaughtering them or detaining them in isolation camps. Such a measure would, however, entail the provision of a staff of trained microscopists, as animals often have large numbers of parasites in the blood, and are therefore highly infectious, some time before the appearance of symptoms; and the herdsmen themselves are accustomed to slaughter their stock

as soon as they show definite signs of sickness. Camps also should be instituted near every large town and European station, in which sick horses, donkeys, and any other animals that it was inadvisable to slaughter, might be isolated.

Great importance has recently been attributed to the *rôle* played by big game in the spread of trypanosomiasis. It should undoubtedly be determined what forms of trypanosome they harbour in Northern Nigeria; but before undertaking extensive measures to drive back the game from the inhabited areas some experiment on a large scale should be carried out on the lines suggested by Dr. Yorke. It is perhaps unnecessary to insist that, in view of the discoveries of Kinghorn and Yorke in Rhodesia, the destruction of big game in the vicinity of native towns and European stations should be encouraged instead of being artificially restricted.

I have to thank Mr. P. M. Dwyer and Mr. E. C. Duff, the Residents successively in charge of the province, and all the officers of the Administrative Department at Ilorin for their hearty co-operation, and especially Mr. R. Scott Chapman, Mr. T. A. G. Budgen, and Mr. C. S. Burnett, who when on tour in their respective divisions, and in addition to their arduous duties, undertook the supervision of natives trained by me to collect biting flies. Their assistance brought within my reach even the most remote parts of the province, and enabled me to obtain specimens from practically every inhabited district. I am also indebted to Mr. Budgen for very kindly taking the photographs which illustrate the tsetse-haunts in the eastern division of the province, and to Mr. O. Vetter for permission to reproduce his photograph of Eri.

With but few exceptions the identifications of all the tsetse-flies in the collections referred to in this paper have been confirmed by Mr. Guy A. K. Marshall to whom I wish to tender my most sincere thanks both for carrying out this tedious and difficult work, and for his unfailing kindness and encouragement.

Synopsis of localities in Ilorin province, Northern Nigeria, in which Tsetse-flies were taken during 1912.*

Western Division.

Abankola, Lanwa district	vi.	P.		Agbaku	vii.	T.	
Abeokuta, near Bangbuse	vii.	T.		Agbiyangi, and between Agbiyangi and Akibiori	x.	P.	
Ademu, near Shari	viii.	P., T.		Agbona	vi.	P.	
Adibongo	vi.	P.		Agodi, Small stream near	viii.	T.	
Adio, Pools at	viii.	P.		Agodi, Pools in a stream-bed near	viii.	P.	
Adio, Busamu River near	viii.	P.		Agoki	vi.	P., T.	
Adiosun	vi.	P.		Agorombi	vi.	P.	
Adiosun	vii.	P.		Aitoru. Between Aribi and Aitoru, near the latter	ix.	P.	
Adukensi	vii.	T.		Aitoru	ix.	P.	
Afonkeke, Oke Oyi district	vi.	P.		Ajidungari	ix.	P.	
Afonkeke	vii.	P., T.		Akata	ix.	P.	
Agara or Agura	ix.	P.		Akata, Oyun River near	ix.	P.	
Agbabiaka, Yalu River near	ix.	P.					
Agbadamu	ix.	P.					

* In this synopsis the following abbreviations are used:—P. for *Glossina palpalis*; T. for *G. tachinoides*; M. for *G. submorsitans*; L. for *G. longipalpis*. The month in which the species was observed is indicated by Roman numerals.

WESTERN DIVISION—*continued.*

Akibiori, and between Akibiori and Babasali ... ... x. P.
Akimano, near Adukensi ... ... vii. P., T.
Aladie, near the Aramu River ... viii. P.
Alapa or Alaqua, Stream a little to the west of ... ... iv. P., T.
Alapa ... ... vi. P.
Alapa. Stream-bed a little beyond the Imoru River on the Ilorin-Alapa Road ... ... viii. T.
Alapa-Ilorin Road. Stream-bed near Alapa ... ... viii. P., T.
Alapa. Stream-bed about 4 miles from Alapa on the road to Ilorin ... ... viii. P.
Alapa. Stream across the road about midway between the Weru River and Alapa ... ... viii. P., T.
Alapa. Near a village a mile or so from Alapa on the road to Oniri ... ... viii. T.
Alapa. Dry river-bed a little nearer Oniri ... ... viii. P., T.
Alapa. Marsh still further on towards Oniri ... ... viii. P.
Alaro, River near ... ... viii. P.
Alaro ... ... viii. T.
Alatiko ... ... vii. P.
Alega or Elega ... ... vii. P.
Aleri, Weru River near ... ... vi. P.
Aleri ... ... vi. P.
Aleyegun ... ... viii. P., T.
Alikun ... ... vi. P.
Aniya ... ... ix. P.
Anwa River, between Moma and Agodi ... ... viii. P.
Anwa River, between Oloru and Agodi ... ... viii. P., T.
Anwa River, between Shao and Oloru ... ... viii. P.
Anwa River, to the north of Shao viii. T.
Apada ... ... viii. P.
Apata ... ... ix. P.
Apola ... ... ix. P.
Arabadi ... ... vii. T.
Aramu River, in Agodi district viii. P.
Aribi, and half-way between Aribi and Aitoru ... ... ix. P.
Ariore. Near the wells ... ... vii. P.
Ariore ... ... ix. P.
Asekweri, Weru River near ... viii. P.
Asomo ... ... viii. P., T.
Asunara ... ... vii T.
Awodi, Awe River near ... ... viii P.
Awodi ... ... viii. P.
Awunyen ... ... vii. P.
Aza River, one mile south of Ilorin railway station ... ... iv. P., T.
Aza River, just below Sobe rock, Ilorin (Pl. III., fig. 2) ... vii. P.
Babadudu. In thick scrub about ¾ mile from Babadudu on the Ariore road ... ... vii. P.
Babadudu ... ... ix. P.
Babagba, Anwa River near (Pl. VI., fig. 1) ... ... viii. P., T.
Babagba, and between Babagba and Olororo ... ... x. P.
Babasali ... ... x. P.
Babanloma ... ... vii. P., T.
Babanloma, A mile or two to the west of ... ... vii. P.
Bachitta, and on stream half-way between Bachitta and Pandaregi x. P.
Balogun, Stream a little to the north of ... ... vi. P.
Balogun. In the kurumi behind the town; no water ... ... vi. P.
Balogun, Stream to the west of, about half-way between Balogun and the Oshin River ... ... vi. P.
Balogun, Stream on the Agugi-Ilorin road a little to the west of ... ... x. P.
Balogun ... ... x. P.
Balogun, Stream on the Agugi-Ilorin road a little to the east of ... ... x. P.
Balogun, Ogbe River near ... x. P.
Bangbuse ... ... vii. P., T.
Banseriki, between Lanwa and Ilorin ... ... iv. P.
Barre, at the Oyi River near ... x. P.
Barre. Between Barre and the hill near Oroki ... ... x. P.
Bemigun ... ... vii. T.
Bina or Odobina ... ... vi. P.
Biribiri ... ... ix. P.
Bode Sadu. Oshin River at Bode Sadu railway station ... ... vii. P.
Bongbo or Kongo River, a few miles from Paiye on the road to Oniri ... ... viii. P.
Bude Egba ... ... iv. P., T.
Bude Egba ... ... v. P., T.
Budo Oya Deogun ... ... ix. P.
Budori ... ... viii. T.
Budu River ... ... vii P., T.

Western Division—*continued.*

Buduia River ... ... viii. P.
Busamu ... ... viii. T.
Busamu River, near Guduaga ... vii. T.
Busamu River, near Adio ... viii. P.
Dagbuana, Elejula River between Paiye and ... ... viii. P.
Dagbuana, Hamma River near ... viii. P.
Dogari, Imoru River between Awodi and ... ... viii. P.
Dogari or Dongari, Stream (Ala) near ... ... viii. P.
Dumagi. In a kurumi just behind the village ... ... iii. P.
Dumagi, Stream between Shari and x. P.
Dumagi ... ... x. P.
Efue ... ... viii. P.
Egwo River, between Lanwa and Ariore ... ... vii. P.
Elegberun, near Ajidungari ... ix. P.
Elehula, near Elega ... ... viii. P.
Eleja, near the Aramu River ... viii. P.
Elejula River, between Paiye and Dagbuana ... ... viii. P.
Elelemezi, near Aramu ... ... viii. P.
Elemeri ... ... ix. P.
Famali. Stream near Famali on the road to Shari ... ... x. P.
Famali. Stream half-way between Shari and ... ... x. P.
Fata, and on Awhong River near Fata ... ... vi. P.
Fata, *Ruga* at ... ... viii. P., T.
Fata. Elebue River, a tributary of the Awhong River, 3 miles south of Fata ... ... viii. P., T.
Fata. Elefura Stream, a tributary of the Weru River, 2 miles N.W. of Fata ... ... viii. P., T.
Fata. Onibongbo River, tributary of the Awhong River, near Fata ... ... viii. P., T.
Fata. Awhong River on the road between Elega and Fata ,,, viii. P.
Gabe, near Lanwa... ... ... vi. P.
Gambari, near Pako ... ... ix. P.
Geruwu, and on stream near Geruwu ... ... viii. P.
Geruwu, Stream half-way between Sokoto and ... ... viii. P.
Gori... ... ... vii. T.
Guduaga ... ... vii. T.
Gunia, Pools of a stream near ... viii. P.
Gunia, Dry bed of a stream near (to N.W.) ... ... viii. P.
Gwandegi ... ... x. P.
Hamma River, near Dagbuana ... viii. P.
Ibada ... ... vii. P., T.
Idianya ... ... vi. P., T.
Idiko ... ... viii. P., T.
Idiya, and on stream near ... vii. P.
Idula ... ... vii. T.
Igbeode ... ... vii. T.
Igbaja, Asseya River near ... ix. P.
Igbaja, Malete River near ... ix. P., T.
Igbaja ... ... ix. P.
Igbamalaba ... ... vii. P.
Igbo River. A stream a little on the Weru side of Oke Barbar Hills ... ... viii. P.
Igbokedu or Bokedu, half-way between Balla and Wara ... viii. P.
Igporin, Stream half-way between the Oshin River and ... ... iv. P.
Igporin ... ... viii. P., T.
Igrunia, near Igbeode ... ... vii. T.
Ilanyamyamo, near the River Imoru, on the road from Ilorin to Awunyen ... ... vii. P., T.
Ilayetu ... ... vii. T.
Iliapa. Stream on the Agugi-Ilorin road between Iliapa and the Oyun River... ... ... x. P.
Iliapa. Stream on the Agugi-Ilorin road a little to the west of Iliapa ... ... x. P.
Iliapa ... ... x. P.
Iliapa. Stream on the Agugi-Ilorin road just to the east of Iliapa ... ... x. P.
Ilonwyi ... ... vii. P., T.
Ilorin, Oyun River near (Pl. I, fig. 2) ... ... iii. P., T.
Ilorin. Oyun River at the point where it is crossed by the Patigi-Ilorin road ... ... iv. P., T.
Ilorin. Oyun River near Ilorin on the Patigi-Ilorin road ... iv. P., T.
Ilorin, Oyun River near ... ... viii. P.
Ilorin. Foma River, just behind the town, in an isolated clump of trees to one side of the road viii. P.
Ilorin. Imoru River at the crossing on the road to Alapa... viii. P.
Ilorin. Oyun River near Ilorin on the Agugi-Ilorin road ... x. P.
Iluma ... ... viii. P., T.
Imoru River, just behind Ilorin town ... ... iv. P., T.

WESTERN DIVISION—*continued.*

Imoru River, on road to Ojubede vi. P
Imoru River, on road from Ilorin to Idiko ... ... viii. P., T.
Indachi ... ... x. P.
Iwa River, on the road to Old Oyo ... ... viii P.
Jato... ... ... ix. P.
Jebba ... ... vii. T.
Jebba ... ... ix. P.
Jebba. Near Mr. Fermin's house ix. P.
Jebba, Oya River near ... ... ix. P.
Jebba. Hill called Oke Kungun, or Oke Kessa ... ... ix. P.
Jebba, a couple of miles to the S.E. of (Pl. V, fig. 1) ... ... ix. P.
Jodomo. At the railway station vii. P.
Jodomo. Dry stream-bed near the railway line... ... ... vii. P.
Jodomo ... ... ix. P.
Konkofu ... ... iii. P., T.
Kulendi district. About 1½ miles N. of the Ilorin Residency along the railway line ; a little on the Oyun River side of the line ... vii. P.
Kulendi district. About 2½ miles along the railway line from the Residency towards Oyun River station ... ... vii. P.
Kulendi district. In the bush about midway between the railway line and the Oyun River, some 3 or 4 miles from Ilorin towards the north ... vii. P.
Laiere or Ilaiere ... ... viii. T.
Lanwa railway station ... ... vi. P.
Lanwa ... ... vi. P.
Lanwa ... ... ix. P.
Lanwa, a little to the north of ... ix. P.
Malete ... ... viii. P., T.
Malete, between Ologede and ... viii. P.
Malete. Apoya River, a tributary of the Busamu River, 3 miles east of Malete ... ... viii. P.
Malete, Busamu River east of ... viii. P., T.
Malete. Aui River, tributary of the Busamu River, near Malete viii. P., T.
Maluma. *Ruga* near Maluma, between Oloru and Agodi ... viii. T.
Mokolo ... ... x. P.
Moma, pools of a stream near ... viii. T.
Moni, near Agodi ... ... viii. T.
More, Oyun River just below ... viii. P.
Nafamu River, between Shao and Oloru ... ... viii. P.
Nafamu River, on the road to Oloru ... ... viii. P.
N'dafa River, between Shonga and the River Oshin... ... ... iv. P.
Noma, near Ajidungari ... ... ix. P.
Nquanda ... ... viii. T.
Oba River, just before it flows into the Oyun River ... ... vii. P.
Obada ... ... v. P.
Obaniswa, and on River Nigia or Egia near Obaniswa ... ... viii. P.
Odo Agba ... ... ix. P.
Odo Eri, Kulendi district ... ix. P.
Odo Oba, near the Aramu River... viii. P.
Odorogun ... ... ix. P.
Odumbaka ... ... viii. P., T.
Odumbaka, on road from Alaro to ... ... viii. P.
Ogatiye ... ... x. P.
Ogatiye, half-way between Agbiyangi and ... ... x. P.
Ogidi ... ... vi. P.
Ogundele ... ... v. P., T.
Ogudu ... ... iii. P.
Ojubede ... ... vi. P., T.
Oke Agba ... ... ix. P.
Oke Dari ... ... ix. P.
Oke Maifura ... ... viii. P.
Oke Oyi, between Oke Magura and ... ... iv. P.
Oke Oyi ... ... viii. P.
Okitepo, between Igbokedu and Wara ... ... viii. P.
Oloda, near Ladan ... ... ix. P.
Olofaganga, near Ajidungari ... ix. P.
Ologbin ... ... viii. P.
Ologede ... ... viii. P.
Ologogi ... ... vii. P.
Ologogi River ... ... viii. P.
Olororo ... ... x. P.
Oloru, Alako River near ... ... viii. P.
Oloru, Olori River two miles to the S.W. of ... ... viii. T
Oloru, dry stream-bed a mile or two south of ... ... viii. P.
Oloru. Small stream, a tributary of the Anwa, between Oloru and Agodi ... ... viii. T.
Omatuntu. Omogo River (pools only) near Omatuntu, on the far side of Oke Barbar... ... viii. P.
Onimaji or Animaji ... ... viii. P., T.
Oniri. Weru River near Oniri and to the west ... ... viii. P.

Western Division—*continued.*

Oniri, banks of the Awhong River, E. of ... ... viii. P.
Oniri. Pools in the bed of a large stream on the Alapa-Oniri road viii. P., T.
Oniri. Stream-bed near Oniri on the Paiye-Oniri road ... ... viii. P.
Oniri. Dry bed of a stream between Paiye and Oniri ... ... viii. P.
Oniwara River, on the road to Budo Illa, Kulendi district ... vi. P., T.
Oqualaquala, under a big tree between Ibaba and ... ... vii. T.
Oqualaquala ... ... vii. P., T.
Oreri ... ... ix. P.
Oreri, stream to north of ... ix. P.
Oroki, hill between the Oyi River and ... ... x. P.
Oroki, and on stream near Oroki x. P.
Oroki, between Ogatiye and ... x. P.
Oshin. At the River Oshin on the Patigi-Ilorin road ... ... iii. P.
Oshin River, where the Patigi-Ilorin road crosses it ... ... iv. P.
Oshin River, where it crosses the Balogun-Ilorin road ... ... iv. P., T.
Oshin River, on the Agugi-Ilorin road ... ... vi. P.
Oshin River. Small stream, a tributary of the Oshin River, on the Agugi-Ilorin road, a little to the W. of the Oshin River ... ... vi. P.
Oshin River, where the Shonga-Jebba road crosses it (Pl. VI., fig. 2) ... ... iv. T.
Oshin River, just south of Bode Sadu ... ... ix. P.
Oshin River, one mile south of Bode Sadu ... ... ix. P.
Oshin River, three miles south of Bode Sadu ... ... ix. P.
Oyi River, where it crosses the Agugi-Ilorin road ... ... iv. P.
Oyi River, on the Agugi-Ilorin road ... ... vi. P.
Oyi River, about one mile north of the Agugi-Ilorin road ... x. P.
Oyi River, near Igbaja ... ... ix. P.
Oyi River. Bakin Gulbi, on main road between Shari and Zumbufu ... ... viii. P.
Oyi River, near Zumbufu ... viii P., T.
Oyun River, near Ilorin (Pl. I. fig. 2) ... ... v., vi., vii P., T.
Oyun River, on Patigi-Ilorin road, near Ilorin ... ... vii. T.
Oyun River, near Ilorin, near main caravan road ... ... vii. P.
Oyun River, near Ilorin. Reported by the collector to have been caught on a *chedia* tree (*Ficus sp.*) ... ... x. T.
Oyun River, where it crosses the Balogun-Ilorin road ... ... iv. P., T.
Oyun River, on the Agugi-Ilorin road ... ... vi. P.
Oyun River. Small stream to the W. of the Oyun River on the Agugi-Ilorin road ... ... vi. P., T.
Oyun River, just beyond the stream Oniwara ... ... vii. P.
Oyun River, half-way between the streams Oniwara and Oba ... vii. P.
Oyun River, a little to the N. (towards the River Anwa) of the Patigi-Ilorin road ... ... vii. P., T.
Oyun River. About ½ mile further along the river in the same direction ... ... vii. P., T.
Oyun River. About ¼ mile further on in the same direction ... ... vii. P.
Oyun River. About ¼ mile further on in the same direction vii. T.
Oyun River. About ½ mile further on in the same direction vii. P., T.
Oyun River. About ¼ mile further on in the same direction vii. P.
Oyun River. To the west of the railway bridge over the Oyun River close to Oyun River Station ... ... ix. P.
Paiye ... ... viii. P.
Paiye. Stream between Paiye and the Weru River on the Paiye-Malete road ... ... viii. P.
Paiye. Onikonko River 2 miles to the N. W. of Paiye ... ... viii. P.
Paiye. Stream between Paiye and Oniri ... ... viii. P.
Pako. Oni Pako ... ... ix. P.
Pako. At the new Rest-House on the road to Jebba a little way from Pako ... ... ix. P.
Pako, between Jebba and ... ix. P.
Pandaregi, stream on the road between Bachitta and ... ... x. P.
Pandaregi ... ... x. P.

Western Division—*continued.*

Pandaregi. Ndafa River on the road from Pandaregi to Shari...	x.	P.
Pasa River	viii.	P.
Patiko, and on stream between Patiko and Indachi	x.	P.
Popo, near Awodi...	viii.	P.
Sapata	x.	P.
Shao, River Anwa near	iii.	P.
Shao, River Imoru near	iii.	P.
Shari or Sharagi	vii.	P.
Shari	viii.	P.
Shari	x.	P.
Shari. Hill behind the town ...	x.	P.
Shari. Pool near Shari on the road to Dumagi...	iii.	P.
Shari. Stream half-way between Shari and Dumagi	iii.	P.
Shari. Stream between Shari and Dumagi	x.	P.
Shari. Kurumi on the road to Lafiagi	x.	P.
Shari. Stream on the Shari-Famali road, nearer Shari ...	x.	P.
Shonga. At a stream just behind the town	iii.	P.
Shonga. On the fringe of a clump of trees at the margin of the lagoon just outside the town ...	iii.	P., T.
Shonga	iv.	P., T.
Shonga, and on the river which flows from Dumagi to Shonga...	x.	P.
Sokoto	v.	P.
Sokoto, Pasa River near	viii.	P.
Sokoto. Stream beyond Sokoto towards Awodi	viii.	P.
Umaso	vii.	P., T.
Wara	viii.	P.
Waro Olope	viii.	P.
Weru River (near), to the N.W. of Agodi district	viii.	P.
Weru River (near), in the N.W. corner of Agodi district ...	viii.	P.
Weru River, on road between Gunia and Laoli; at present only pools. (Pl. II., fig. 1.) ...	viii.	P.
Weru River, where the Paiye-Malete road crosses it	viii.	P., T.
Weru River, between Oniri and Paiye	viii.	P.
Weru River. Collection made at various points along the Weru River between the crossing on the Oniri-Paiye road and the junction with the Awhong River	viii.	P.
Weru River, at the crossing on the road from Paiye to Alapa ...	viii.	P., T.
Yaru. Small stream, a tributary of the Oyi River, to the E. of Yaru	vi.	P.
Yaru, Small stream to the W. of	vi.	P.
Yaru, Oyi River beyond	vi.	P.
Yaru, Small stream between Balogun and	vi.	P.
Yaru	x.	P.
Yaru, Stream a little to the E. of	x.	P.
Yuregi, At a *ruga* near	viii.	P.

Eastern Division.

Agugi, Valley ½ mile to S.W. of	iv.	P.
Agugi, Ogu River ½ mile to S.E. of	iv.	P.
Agugi. Ogu River, a tributary of the Oyi River, near Agugi ...	iv.	P.
Agugi	vi.	P., T.
Agugi. River just behind the village	vi.	P.
Agugi. Stream to the W. on the Ilorin road	vi.	P.
Agugi. Small stream a little to the E. of the Oyi River ...	vi.	P.
Agugi	x.	P.
Ahun, on Oro River	x.	P., T.
Ajikuami, and also a little to the S. of Ajikuami	x.	P.
Arido River, between Egboro and Egbe	vii.	M.
Babanzauri	vi.	P.
Bada	x.	P.
Badokin	x.	T., M.
Bongbo, and between Bongbo and Oke Odde	x.	P.
Bongi. Kuyi River near Bongi on the Patigi-Ilorin road	iv.	P.
Busa, Kurumi by the Kussuko River near	v.	P., T., M.
Busa, Kussuko River near ...	v.	P., T.
Busa, Oyi River near	v.	P., T.
Checkela, Nyemi River near ...	x.	M.
Daji. Stream near Daji on the Rogun-Patigi road	v.	P., T.
Daji	x.	M.
Duro, near Pada	—	P., T., M.
Eche River, near Patigi	v.	P.

Eastern Division—*continued.*

Edogi Chapaka, Niger flats W. of	v.	P., T.
Edogi Chapaka, Niger flats near to (not near water)	v.	P., T., M.
Edogi Pasanako. Edge of Niger flats	v.	P., M.
Egbe	vii.	P., M.
Egbe	viii.	P.
Egbom, Niger River near ...	v.	T.
Egboro	vii.	P., M., L.
Egboro	viii.	P., M.
Egboro	x.	T., M.
Egboro, stream three miles south of	x.	M.
Egboro. Between Egboro and Egbe, two miles N. of the Noako River	x.	P., M.
Ejiba	vii.	M., L.
Emoworigi, near Lattai	x.	P., M.
Emoworigi, Between Kussoko and	x.	P., T., M.
Ere	vii.	M.
Ere. Caught where cattle were grazing	x.	P.
Eri (Pl. IV.)	vii.	T., M., L.
Eri	viii.	T., M.
Erufu	v.	P.
Erufu, between Patigi and ...	v.	P., T.
Erufu	vii.	M.
Erufu	x.	P., T., M.
Erufu, between Egboro and ...	x.	M.
Etchi	vii.	P., M.
Etchi, between Fei and	vii.	P., T., M.
Famali, at the Oye River near ...	iv.	P.
Famali	x.	P.
Fei	vii.	P., M.
Gada	v.	P., T., M.
Gada, between Erufu and ...	v.	P., T.
Gaji. Near Gaji on the Rogun-Patigi road	v.	P.
Galogi	vii.	P., M.
Galogi, and on Nyemi River near Galogi	x.	P., T., M.
Galogi, between Erufu and ...	x.	P., M.
Gbodu	viii.	T., M.
Godiwa, near Patigi	v.	P., T.
Gudu Zuru, between Lafiagi and	v.	P., T.
Idorfin, Kabba Province	vii.	P., T., M.
Isanlu (Oke Oloke), between Lata and	v.	P.
Jijikuo, between Pada and ...	v.	P., T.
Jijikuo, between Rogun and ...	x.	T., M., L.
Kasagi	vii.	M.
Katsido	x.	P., M.
Koro	vii.	P., M., L.
Kusofini, on the Oke Odde-Lafiagi road	iv.	M.
Kusogi Danshi, between Patigi and	v.	T.
Kussuko River	v.	P., T., M.
Kussuko River, between Zumbufu and Lafiagi	vii.	T.
Kuyi River, five miles south of the main Patigi-Ilorin road ...	v.	P.
Labaka, a little to the E. of ...	x.	P.
Labaka, and again a little to the W. of Labaka	x.	P.
Lade, Duku River on the main road near	iv.	P., T.
Lade, stream one mile to the W. of...	v.	P., T.
Lade, three miles south of ...	v.	P., T.
Lade	viii.	P., T., M.
Lafiagi, swamp in Egua valley ...	iv.	P., T.
Lafiagi	v.	P.
Lafiagi, Egua River near... ...	v.	P., T.
Lafiagi, stream between the Kussuko River and	v.	T.
Lafiagi, ten miles to the west of	v.	P., T.
Lafiagi, Niger flats five miles to the N.W. of	v.	P.
Lafiagi	vi.	P.
Lafiagi	viii.	P., T., M.
Lab, swamp opposite to	v.	P.
Lah, edge of the Niger flats opposite to	v.	P., T.
Lalagi, between Lade and ...	iv.	P., T., M.
Lalagi, between Ruganagaji and ...	iv.	P., T., L.
Lalagi	vi.	P.
Lalagi. Odo Oni Pako, the water of Lalagi, on the Lade road ...	viii.	P., T.
Lalagi	viii.	P., T., M.
Lata, between Lade and	v.	P., T.
Lata...	v.	P., T.

EASTERN DIVISION—*continued.*

Lattai	x.	T., M.
Likpata	v.	P.
Magi, Pada district	vii.	T., M.
Mamba	x.	T., M.
Matokun	viii.	P., M.
Matokun, and on Kussoko River near Matokun	viii.	M.
Matokun	x.	P., T., M.
Matokun, between Egboro and ...	x.	M.
Matokun, between Oke Oluchi and	x.	M.
Matokun. In a typical kurumi ...	x.	M.
Michian Gidda, between Zumbufu and Lafiagi	viii.	T., M.
Morrufugi, by R. Nalli, 4½ miles E. of Patigi	v.	M.
Morrufugi	x.	T., M.
Ndeje, near Lade	viii.	T., M.
Ndeliman, on Oke Odde-Lafiagi road; not on a river bank ...	iv.	P.
Noako River, between Egboro and Egbe	vii.	P., T., M.
Noako River. Tsetses very plentiful here in the dry season in the river bed, and particularly in the bushes which overhang the pools of the river. Buffalo graze along the banks in the dry season. Tsetses not so numerous in the wet season ...	x.	P., T., M.
Obala	vi.	P., M.
Odo N'la near Eri	viii.	T., M.
Odo Okeri, stream five miles from	x.	P., T.
Odo Okeri. Kurumi at the Rest House	x.	P.
Odo Okeri. Caught where cattle were grazing	x.	P.
Ofa Ora	vi.	P., T., M., L.
Ogu River, between Ora and Agbeoku	iv.	P.
Oke Odde	x.	P.
Oke Odde, stream to west of ...	x.	P.
Oke Odde, half-way between Labaka and	x.	P.
Oke Oloke, on the trade-route from Patigi to Awtun	v.	P., T.
Oke Oloke (Sanlu), four miles to the N.E. of	v.	P., T.
Oke Oluchi	x.	M.
Oke Oluchi, in sparse bush on top of hill at	x.	P., T., M.
Oke Oluchi, on banks of running stream near	x.	M.
Oke Onio	vi., x.	T.
Ologoma	x.	P.
Ora	vi.	P., T.
Ora	x.	P.
Ora, Awere River between Bongbo and	x.	P.
Oro	vi.	P., T., M.
Oro	x.	T.
Owa	vi.	P.
Pada, stream between Jijikuo and	v.	P.
Pada	vii.	T., M.
Patigi. Eche River near the Patigi Residency on the Patigi-Ilorin road	iv.	P., T.
Patigi, two streams one and three miles to the E. of	v.	P., T.
Patigi, one mile to the E. of ...	v.	P.
Patigi, tributary of the Kuyi River near	v.	P.
Patigi, swamp on the road to Lah near	v.	P.
Patigi	viii.	P., T M.
Patigi, Niger bank at	viii.	P.
Patigi. Stream between the Residency and the town	viii.	P.
Patigi, Malalase stream near ...	viii.	P., T.
Patigi, Nyemi River near ...	x.	M.
Reke	vi.	P.
Rogun, and also south of the Kampe River near Rogun ...	v.	P., M.
Rogun	vii.	T., P.
Rogun	viii.	T., M.
Rogun, Kampe River near ...	viii.	P., T., M.
Rogun	x.	P., T., M., L.
Rogun, between Daji and ...	x.	P., M.
Ruganagaji, west of Lafiagi, in the Egua swamp	iv.	P., T.
Sakpefu, edge of the Niger flats near	v.	P., T.
Sanlu, Kabba Province	vii.	M.
Sukugi	vii.	P., T., M.
Sula, between Magi and Kasagi ...	vii.	M.
Susonogi, Niger swamp between Gakpan and	v.	P., T M.
Tampa-balogun, Stream between Lafiagi and	iv.	T.
Tsuvun, between Pada and ...	vii.	P., T.

Eastern Division—*continued.*

Tutugi, stream on the Rogun-Patigi road	v.	P., M.	
Tutugi	x.	P., M.	
Yabagi or Yagbagi	vii.	P., T., M.	
Yamu Zuru, near Niger swamps	v.	P., T., L.	
Yelua, near Lafiagi	viii.	P., T., M.	
Zongo, near Lafiagi	viii.	P.	
Zumbufu	vii.	T., M.	
Zumbufu	viii.	P., T., M.	

Southern Division.

Afon	v.	P., T.	
Afon	viii.	P.	
Agbamu	ix.	P.	
Ajassepo	viii.	P.	
Akpa	ix.	P.	
Alabi, on the Odo Oyi	ix.	P.	
Amonifaza, near Ilorin	ix.	P.	
Aredube, on the Oshin River	ix.	P.	
Aula	ix.	P.	
Awsun	ix.	P.	
Awtun, between Etan and	v.	P.	
Awtun. In thick bush around the town	vi.	P.	
Aza River, south of Ilorin	viii.	P.	
Edidi	viii.	P.	
Ekan	ix.	P.	
Epetu. Thick bush	vi.	P.	
Erin. Near the railway line	vii.	P.	
Eruku	viii.	P.	
Etan	ix.	P.	
Gama	vii.	P.	
Idiemi	v.	P.	
Idofian, near	ix.	P.	
Idofian, Odo Aweri near	ix.	P.	
Idofian, Okagi River near	ix.	P.	
Idofin	ix.	P.	
Igboma	viii.	P.	
Igbonla. Thick bush	vi.	P.	
Igbowo	ix.	P.	
Ikauton	viii.	P.	
Illa	v.	P., T.	
Illa market	iv.	P., T.	
Illotta	ix.	P.	
Illotta, Igbo Illotta, bush near	ix.	P.	
Iloffa. Thick bush	vi.	P.	
Iloffa	viii.	P.	
Ilorin. On the road to Olobon-doroko	viii.	P.	
Isanlu, along a stream between Omu and	v.	P.	
Isanlu	ix.	P.	
Kanla River, near Obaniswa	viii.	P.	
Laduba	viii.	P.	
Laiyadu	ix.	P.	
Obate	vii.	P.	
Obo	viii., ix.	P.	
Odde, Afon district	viii.	P.	
Odo Eku	ix.	P.	
Odo Jagu	ix.	P.	
Odo Ogbo	ix.	P.	
Odota River, on the Ilorin-Obaniswa road	viii.	P.	
Offa	iii.	P.	
Offa. In the swamp (Pl. III., fig. i.)	iv.	P.	
Offa. Thick patch of bush to the W. of the Residency	iv.	P.	
Offa, Oyun River near	viii.	P.	
Offa. To the W. of the native town	ix.	P.	
Ojuko	ix.	P.	
Oke Aba	ix.	P.	
Oke Awra	iii., viii.	P.	
Oke Illa. At the Illa River	ix.	P.	
Oken Igbe	vi., viii.	P.	
Okeya. In thick bush round Okeya between Ajassepo and Igbadja	vi.	P.	
Okun River, on the road to Obaniswa	viii.	P.	
Olobondoroko	vii., viii.	P.	
Olobondoroko. On the road to Ilorin	viii.	P.	
Omedo	ix.	P.	
Omu	iii., v.	P.	
Omu. Around Omu in thick bush and near a stream	vi.	P.	
Oquale	viii.	P.	
Orimope	viii.	P.	
Oro River, between Idofin and Eruku	vi.	P., T.	
Osale River, on Ilorin-Obaniswa road	viii.	P.	
Osi	v., viii.	P.	
Owo, between the Oyi River and	iv.	P.	
Owo, between Isanlu and	v.	P.	
Sakpe	ix.	P.	

Fig. 1. The cattle that sicken with trypanosomiasis on the way to the coast are slaughtered and laid out for sale on the roadside.

Fig. 2. Oyun River near Ilorin, showing an actual haunt of *G. palpalis* and *G. tachinoides*.

Fig. 1. Weru River in the dry season; a haunt of *G. palpalis*.

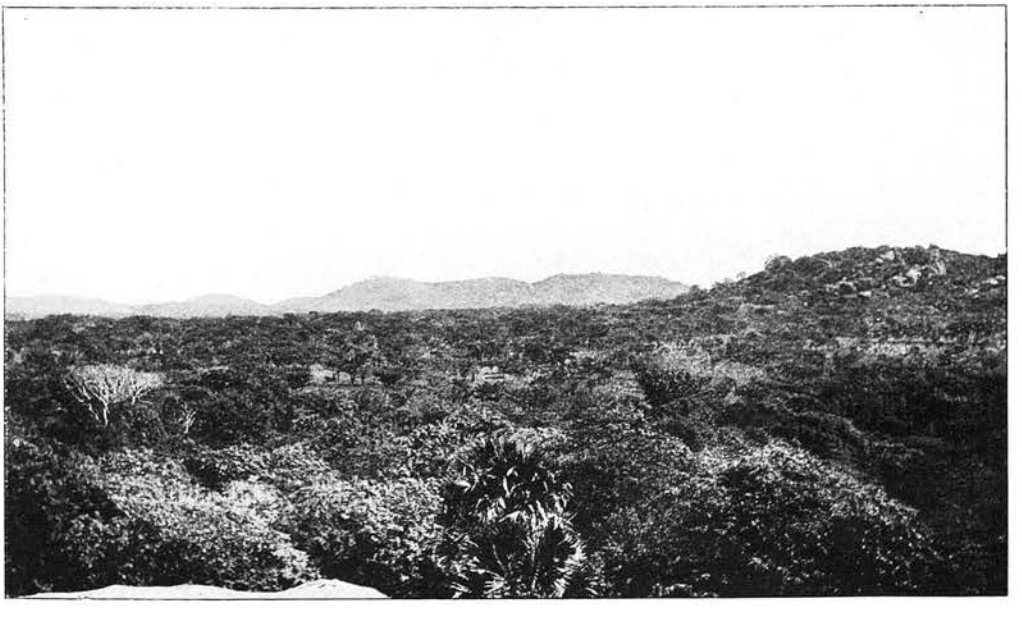


Fig. 2. View from Old Oyo looking towards Ilorin; uninhabited country.

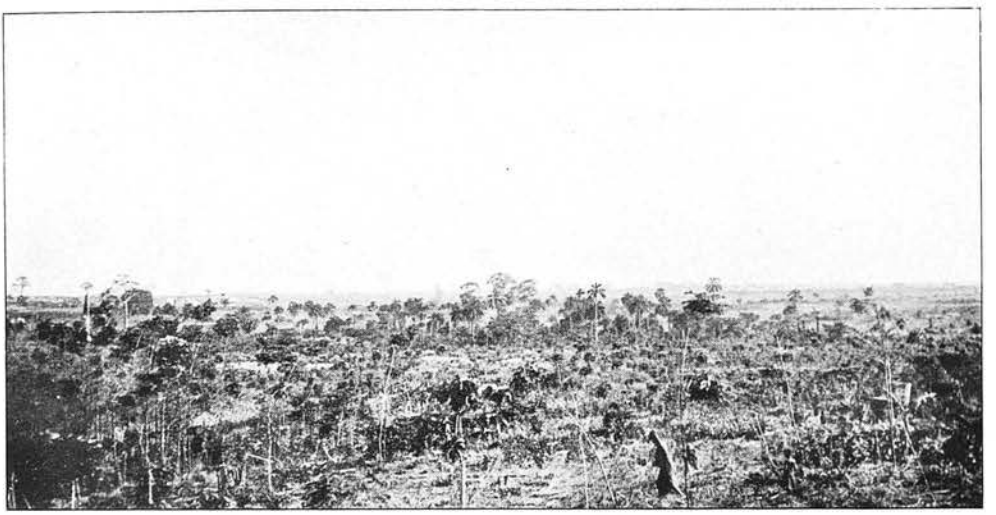


Fig. 1. The fly-belt at Offa; a haunt of *G. palpalis*.

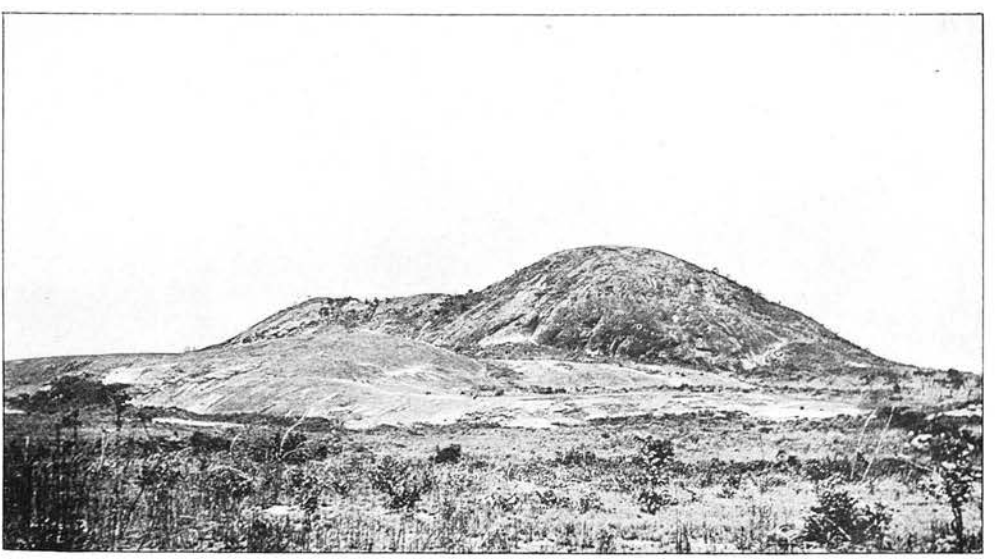
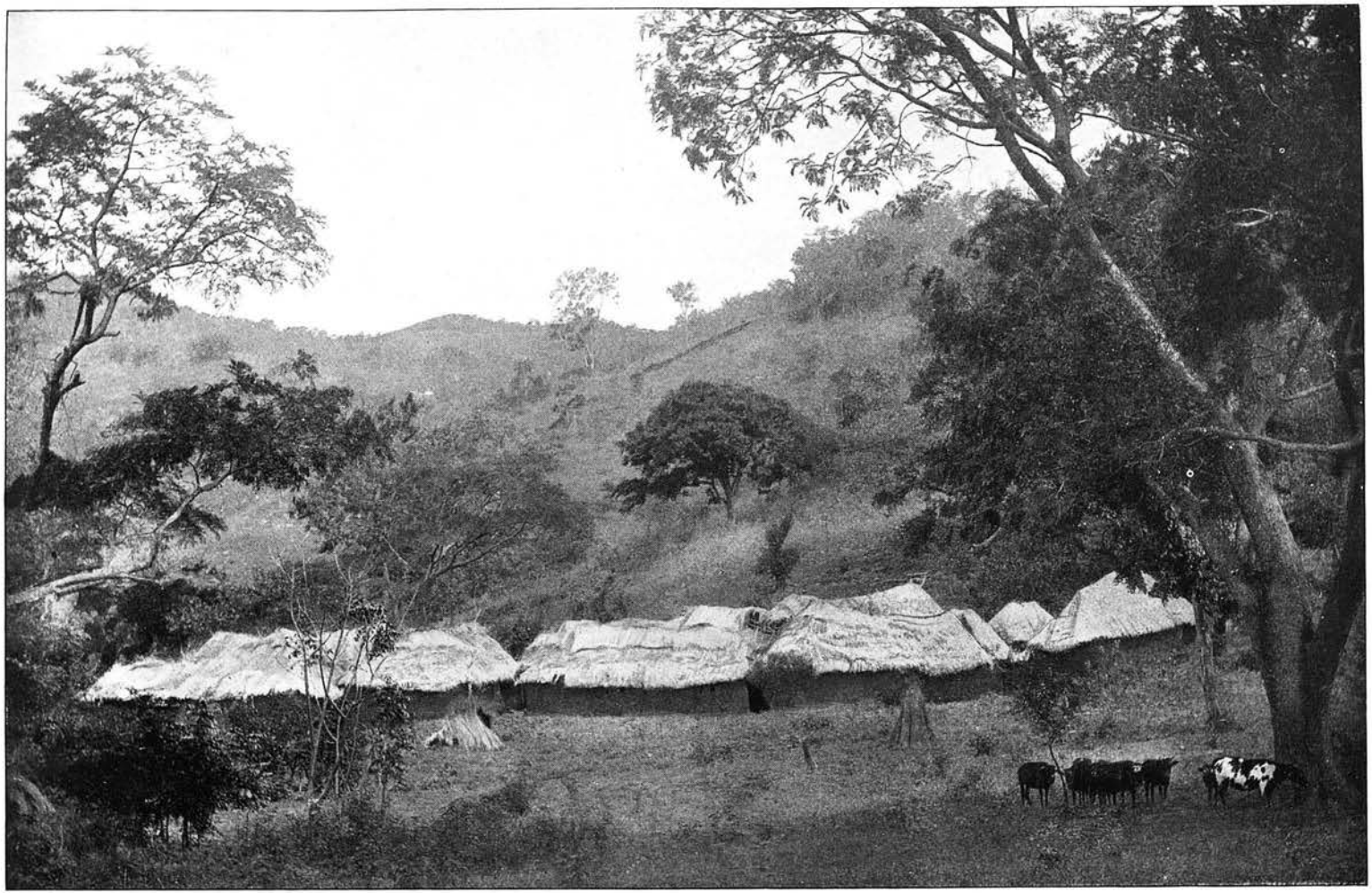


Fig. 2. Sobe Rock, near Ilorin; one of the isolated hills typical of the scenery of Ilorin; *G. palpalis* was taken at the foot of this rock.



The village of Eri: with dwarf cattle in foreground.

Fig. 1. Path through bush near Jodomo Railway-station, frequented by *G. palpalis*.

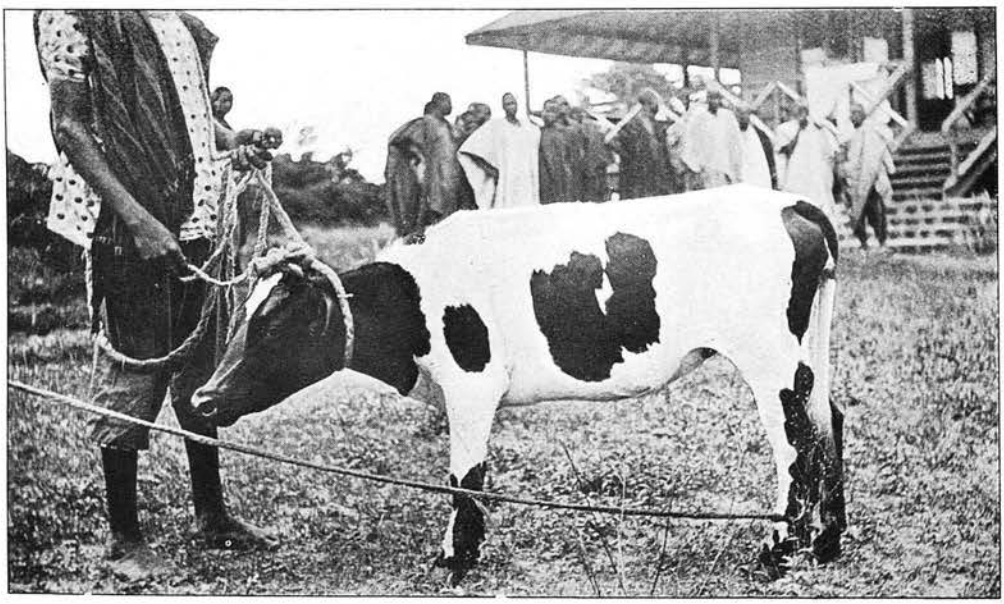


Fig. 2. A cow of the dwarf breed of cattle found in Nigeria.

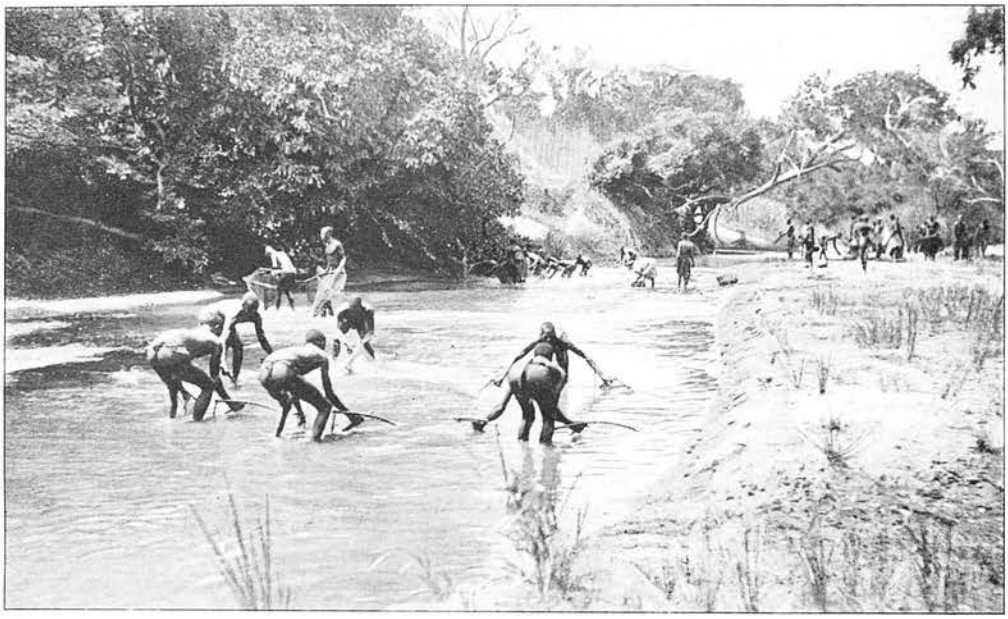


Fig. 1. Natives fishing in the Anwa River at Babagba; a spot where *G. palpalis* and *G. tachinoides* occur.

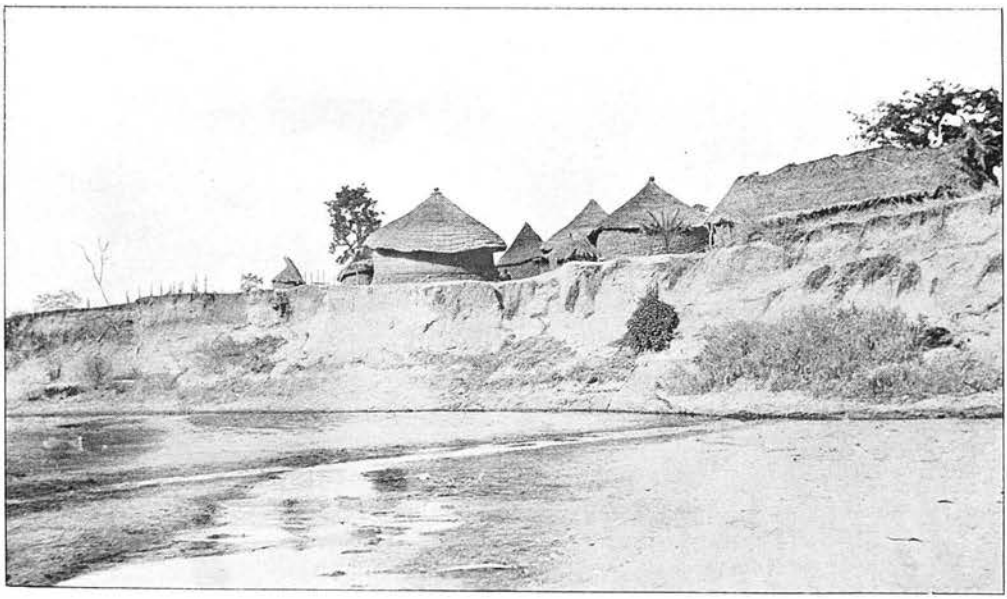
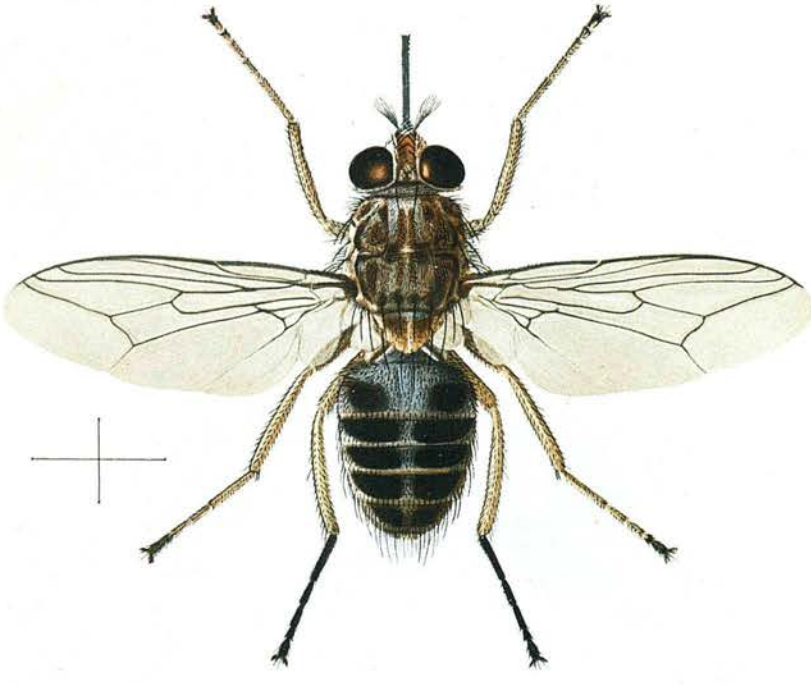
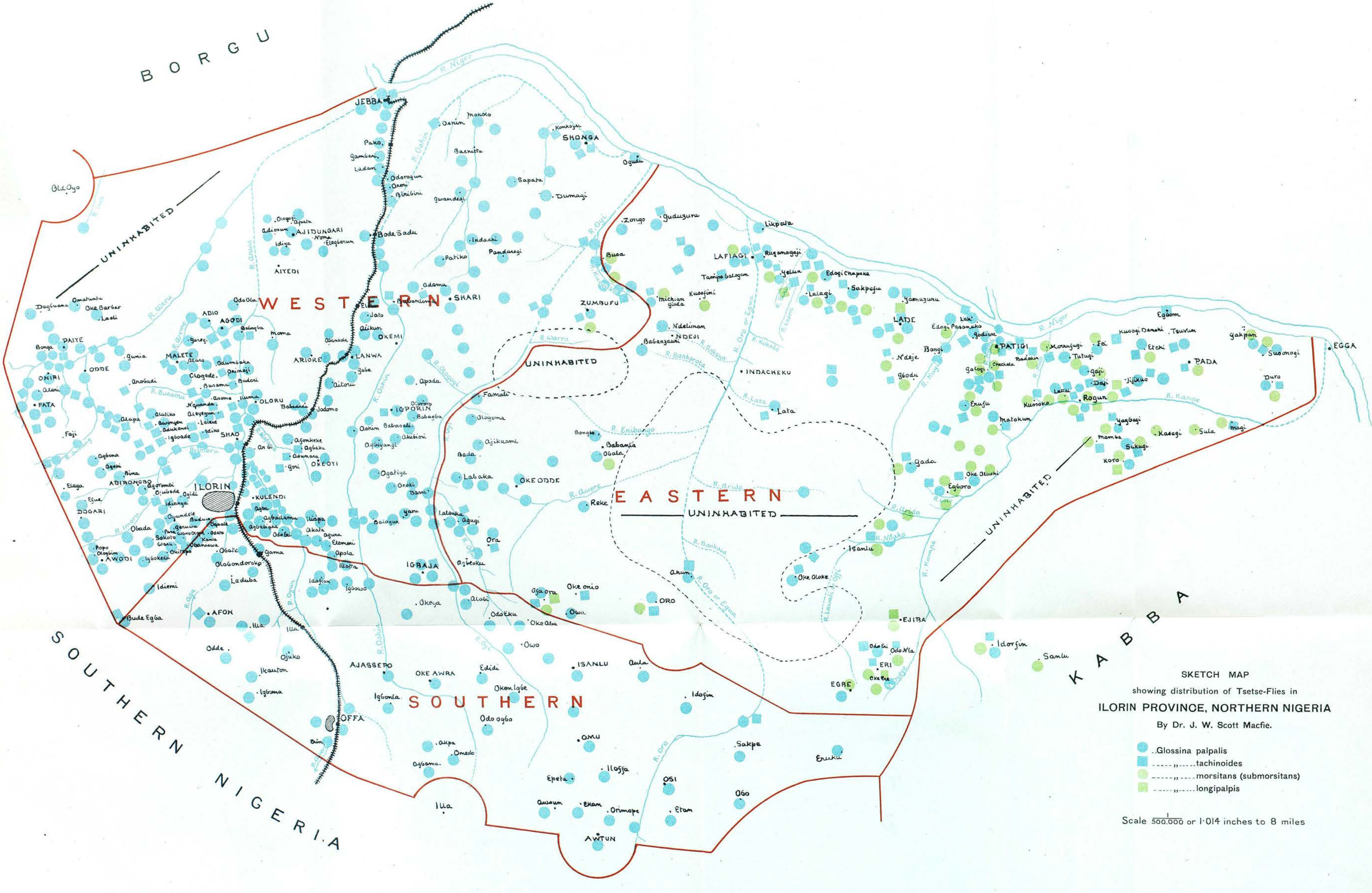


Fig. 2. Oshin River, near Jebba, in the dry season; a haunt of *G. tachinoides*.



A. J. E. TERZI, DEL. BALE & DANIELSSON, LTD.

GLOSSINA PALPALIS, R.D., var.



SKETCH MAP

showing distribution of Tsetse-Flies in

ILORIN PROVINCE, NORTHERN NIGERIA

By Dr. J. W. Scott Macfie.

With the Authors Compliments

ANNALS
Edited by
RONALD ROSS
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J. W. W. Stephens
R. Newstead
J. L. Todd
H. W. Thomas
Anton Breinl
H. B. Fantham

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TRYPANOSOMIASIS OF DOMESTIC ANIMALS IN NORTHERN NIGERIA

BY

J. W. SCOTT MACFIE, M.A., M.B., CH.B.

WEST AFRICAN MEDICAL STAFF

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PLATES I—III

Trypanosomiasis is very prevalent amongst domestic animals in Northern Nigeria, and, at any rate in the south-western portions of the Protectorate, accounts not only for a heavy mortality, but also for serious financial losses, due to sickness, forced sales and the slaughtering of stock in unsuitable markets. It is difficult to give any adequate idea of the grave nature of the losses, but some conception of their extent may be gathered from the two following instances:—During the greater part of 1912 I was stationed in Ilorin, a province which, with the exception of one division, is considered not unsuitable for horses. Nevertheless, during the eight completed months of my residence, ten out of the fifteen horses in the possession of Europeans contracted trypanosomiasis, and six died of the disease. An experiment was also made with a view to introducing animal transport into the province. Ten healthy donkeys were selected for this purpose, and sent to carry loads from Ilorin town to Agugi, a village some thirty miles to the east. Within three months all had died of trypanosomiasis. At Zungeru, during the year 1911, twenty-five horses were treated for this disease in the isolation camp, and of these 40 % either died or were so seriously affected that their owners thought it wise to part with them at the small price a native is always willing to pay for a sick animal. At Lokoja the disease is even more serious, and Dr. C. F. Watson has estimated that among the horses treated for tsetse disease there, the rate of dead and incapacitated horses is over 73 %. There can, moreover, be little doubt that the disease is spreading; a fact proved by the additions made from time to time to the list of localities in which the Government declines to compensate officers for the loss of horses which have died from trypanosomiasis contracted in the discharge of official duties.

Difficult as it is to estimate the losses among horses belonging to the small number of Europeans stationed in the Protectorate, it is altogether impossible to ascertain those of the natives. They must, however, be very great, and their magnitude may be gauged by the experiences of the Emirs who attended the Coronation Durbar at Zaria in June, 1911, one of whom is said to have lost 70 per cent. of his horses. Before the British undertook the administration of Nigeria, certain tracts of country known to be unsuitable for horses were sedulously avoided. Europeans, however, are compelled to penetrate these districts in the discharge of their duties, and are followed by the native officials, not only at the sacrifice of their own animals, but also greatly to the detriment of the whole country, since the infected horses returning home, or passing through other districts, spread the disease in every direction.

Besides horses, the cattle, sheep, goats and dogs are also affected. In their long trek to the coast, the herds of cattle from the north carry trypanosomiasis with them, maintaining the infection in old localities and introducing it into new. The herdsmen are quick to detect the symptoms of the disease, and, if they appear in one of their animals, are accustomed to slaughter the beast wherever they may happen to be. The fate of those that sicken is to be butchered by the roadside, and it is a common sight to see a carcase cut up and laid out for sale far away from any native town. Ilorin is situated at a point on the main caravan road where the routes from Kano and Sokoto converge, and the majority of the animals slaughtered there for the market were found on examination to be suffering from trypanosomiasis.

In Table I the results of the examinations of thirty-five of these animals is shown:—

TABLE I.—Trypanosomes found in the blood of thirty-five sick animals being slaughtered for the Ilorin market

Host	*T. brucei*	*T. vivax*	*T. nanum* or *pecorum*	Double infection
Cattle—Fulani breed	1	18	1	1
Sheep and rams	1	8	1	...
Goats	...	4	...	...
Totals	2	30	2	1

In November, 1911, Sir David Bruce published in No. 31 of the *Sleeping Sickness Bulletin*[1] a list of identifications of fifteen cases collected by me in Northern Nigeria of trypanosomiasis in horses. Since then I have added to these a number of fresh cases, both in horses and other domestic animals, bringing the number up to eighty-eight. An analysis of eighty-six of these cases, in which the infection was natural, is given below (Table II).

TABLE II.—Showing the infecting agent in eighty-six cases of naturally acquired trypanosomiasis in domestic animals in Northern Nigeria

Host	*T. brucei*	*T. vivax*	*T. nanum* or *pecorum*	*T. theileri*	Double infections
Horse	14	18	8	...	3
Donkey	2	2	...	...	...
Cattle—					
Fulani breed	1	18	1	...	1
Dwarf breed	...	2	...	1	...
Sheep and ram	1	8	1	...	...
Goat	...	4	...	...	...
Dog	...	1	...	...	...
Totals ...	18	53	10	1	4

As it was impossible in Northern Nigeria to carry out systematic series of experiments on animals or to attempt cross immunity tests, the morphological characters of the trypanosomes found in blood-films had to be relied on for identification. By this means it was, of course, impossible to distinguish between the more closely allied species. *T. theileri* was observed once, but with this exception the trypanosomes detected fell naturally into three main groups, and, as individuals of each type had previously been examined by Sir David Bruce, his identifications have been adopted.[2]

*T. brucei.** In twenty cases of natural infection trypanosomes were found which closely corresponded with the type described and figured by Sir David Bruce as *T. brucei*.[3] In four cases (three horses and one donkey) posterior nuclear forms were detected, and

* This trypanosome is probably that for which Prof. Stephens and Dr. Blacklock have recently proposed the name *T. ugandae*. (Proc. Roy. Soc., B, Vol. LXXXVI, pp. 187-191).

in guinea-pigs and rats inoculated from three of these cases they appeared at certain stages of the disease in relatively large numbers. Posterior nuclear forms have been described in *T. equiperdum*, *T. pecaudi* and *T. brucei*, as well as in *T. rhodesiense*. Their occurrence in these cases of trypanosomiasis in Nigeria does not therefore assist in precise identification. Sleeping sickness, however, is either altogether absent or quite uncommon at Zungeru and Ilorin, where the majority of my cases were found. In a number of films the long forms of the parasite were observed to have their posterior extremities peculiarly blunt and almost rectangular. No attempt was made to plot a curve to represent the variation in size of this trypanosome. Great variations were observed from day to day in the relative percentages of short and long forms, as is indicated in the tables given below, where the different forms were enumerated in the case of a horse, and in those of a rat and a guinea-pig inoculated with his blood. (See table on page 5.)

The disease set up by these trypanosomes appears to be extremely fatal. Of the eleven horses and two donkeys suffering from this form of trypanosomiasis of which I have complete records none recovered. In horses the disease seems to occur in two forms, some cases end fatally in two to four weeks, others linger on for as many months. All the cases in which posterior nuclear forms were observed were of the acute type, death occurring within a month of the onset of the disease.

The general symptoms in horses were fever, emaciation, and more or less pronounced oedema of the legs, belly and scrotum. The appetite was irregular, but was often ravenous almost up to the hour of death. In the later stages restlessness was a distressing feature. Inflammation of the eyes was observed in two cases out of fourteen, and a greenish discharge from the nose, coughing up of mucus, and a herpetiform eruption on the upper lip occurred each in one case. The animals presented a dejected appearance in the early stages of the disease, their heads drooped, they dragged their hind legs in walking, moved slowly and with difficulty and were apt to stumble. They were notably insensitive to pain, as for instance, to the prick on the ear necessary to obtain blood for examination. Finally they became so weak and wasted that they could not rise.

Natural infections with trypanosomes of this type were found in the horse, donkey, Fulani cattle, and sheep. The parasite was also successfully transmitted by inoculation to a cow of the dwarf breed found in pagan districts of Nigeria. (See page 10.)

Date, 1912	Horse (No. 35)		Rat		Guinea-pig	
	Forms with long, free flagella	Stumpy and intermediate forms	Forms with long, free flagella	Stumpy and intermediate forms	Forms with long, free flagella	Stumpy and intermediate forms
July 8 ...	24%	76%	...	...	...	...
,, 9 ...	42%	58%	...	...	...	...
,, 10 ...	...	...	Rat injected from horse. (Trypanosomes first appeared in the blood on July 16)		Guinea-pig injected from horse. (Trypanosomes first appeared in the blood on July 18)	
,, 13 ...	29%	71%	...	...	...	...
,, 16 ...	Trypanosomes very scanty		89%	11%	...	...
,, 17 ...	...	...	98%	2%	...	...
,, 18 ...	...	...	52%	48%	15%	85%
,, 19 ...	...	...	15%	85%	40%	60%
,, 20 ...	74%	26%	10%	90%	56%	44%
,, 21 ...	...	...	8%	92%	16·5%	83·5%
,, 27 ...	6%	94%	88%	12%	Died July 21	
,, 28 ...	Died		...	...		
,, 29 ...			...	...		
,, 30 ...			58%	42%		
Aug. 1 ...			85%	15%		
,, 4 ...			69%	31%		
,, 5 ...			Died			

T. vivax. By far the most common form of trypanosome in my series of cases was one presenting the morphological features of *T. vivax*. It occurred in fifty-six of my eighty-six cases, and in fifty-three cases was the only trypanosome present, being but once associated with *T. brucei* and twice with *T. nanum* or *pecorum*.

This parasite was found in the following hosts:—Horse, donkey, Fulani cattle, dwarf cattle of Nigeria, sheep, goat and dog. From the fact that it was found in the dog, it is probable that this trypanosome is the parasite to which Ziemann[4] gave the name *T. vivax*, and not the allied *T. cazalboui* described by Pecaud as occurring in Dahomey in horses. For Laveran and Mesnil say: 'Le fait que *Tr. Cazalboui* ne peut être inoculé ni au singe, ni au chien, ni aux petits rongeurs, permet de distinguer facilement ce trypan. des espèces qui s'en rapprochent au point de vue morphologique, mais qui sont pathogènes pour ces animaux.'[5]

In horses the disease set up by this parasite seems to be mild in Northern Nigeria. Of the fifteen cases of which I have complete records only one died, whereas fourteen recovered. The general symptoms were fever, emaciation and oedema of the legs, belly and scrotum, sometimes well marked, at other times scarcely perceptible. The animals presented a dejected appearance, moved awkwardly and reluctantly, and appeared as though tired. In severe cases they were somewhat insensitive to pain. The appetite was always good. In one case cough was a symptom, and in another nasal catarrh. No affections of the eyes were observed. Three cases were met with in donkeys, and of these two died and one recovered. The case of one of those that died was, however, complicated by a concurrent infection with *T. brucei*. Eighteen cases were found amongst Fulani cattle. Most of them were animals treking down towards Lagos from Kano or Sokoto which had sickened on the road, and were therefore about to be butchered for the Ilorin market. Their native owners believed them to be dying, and were therefore having them slaughtered so as to anticipate death; but, beyond this assertion, I have no data from which to gauge the mortality from the disease in this breed of cattle. Both the dwarf cattle which came under my notice suffering from *T. vivax*, however, recovered. Similarly the eight sheep and rams I studied were animals about to be slaughtered so as to anticipate death, and the same remark applies to the four goats. The one dog in which *T. vivax* was found died.

T. nanum or *pecorum*. In a few cases small trypanosomes 9-12μ in length without free flagella were found in blood-films, and these have been identified as *T. nanum* or *pecorum*. 'It will be

remembered the name *T. pecorum* was introduced by Bruce to cover the group of trypanosomes of which the *T. dimorphon* of Laveran and Mesnil and *T. congolense* are members, and that *nanum* is only distinguishable from *pecorum* by animal experiments.'[6]

Trypanosomes of this type were met with in thirteen cases, in ten alone and in three associated with *T. vivax* or *T. brucei*. Ten of these cases were horses, two Fulani cows, and one a ram. Of the ten cases in horses one had a concurrent infection with *T. vivax* and another with *T. brucei*, leaving eight uncomplicated cases, of which one died, three recovered, and the remaining four had unknown issues.

The number of cases of this variety of trypanosomiasis that came under my notice does not, therefore, enable me to give any general account of the symptoms of the disease in Northern Nigeria. It may, however, be noted that oedema was present only to a slight extent in the cases seen, although lachrymal and nasal discharges were relatively more frequent than in infections with either *T. vivax* or *T. brucei*.

T. theileri. *T. theileri* was observed once in a cow of the dwarf breed found in Nigeria. The animal was feverish for a few days and wasted considerably, but soon recovered from the infection.

The period when trypanosomiasis is most common is undoubtedly the rainy season, which corresponds to the time during which the tsetse flies, which in the dry season retire to the banks of the streams and rivers, are most widely distributed over the country. The accompanying chart of the rainfall and the number of admissions of horses to the isolation camp at Zungeru for the year 1911 illustrates this point, although the number of cases dealt with is too small to form a reliable index. A better proof is afforded by the fact that the natives congratulate themselves at the end of the rainy season that those of their horses which have withstood the disease so far will be safe until the rains recommence. The dry season is also the time of year selected by the herdsmen of the north to undertake their long trek south with their cattle and sheep.

The province of Ilorin in Northern Nigeria is peculiar, inasmuch as, whilst *G. palpalis* and *G. tachinoides* are distributed all over it, tsetse flies of the *morsitans* group—*G. submorsitans* and *G. longipalpis*—are restricted to one, the Patigi or eastern, division. In

my series of cases of trypanosomiasis collected at Ilorin, twenty-four were animals that had never been into the Patigi division, and of these fourteen were infected with *T. vivax*, six with *T. brucei* and four with *T. nanum* or *pecorum*. The Patigi division is shunned by all herdsmen, and it is generally recognised that horses can neither live there nor be taken into the district without contracting tsetse disease. Two horses that had lived for years in Ilorin town were taken to Patigi during my last tour of service.

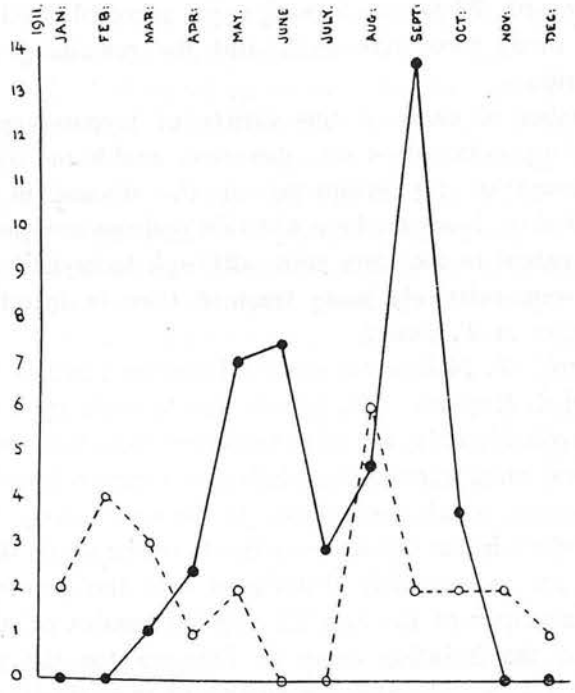

Chart showing the rainfall in inches (continuous line) and the number of cases of trypanosomiasis of horses admitted to the isolation camp (dotted line) at Zungeru during 1911.

Both returned infected with *T. brucei*, and both died shortly after wards. In this connexion it should be mentioned that Bruce concludes that, in Uganda, 'The carrier of *Trypanosoma vivax* is probably *Glossina palpalis*,'[7] and that *G. morsitans* is known to transmit *T. brucei*. It is possible that in the native towns and European stations flies of the genus *Stomoxys*, which abound in these places, may play a part in the transmission of trypanosomiasis. The horse (No. 42), for instance, had not been within

two miles of any spot known to be haunted by tsetse flies for five weeks previous to the onset of his symptoms, and it is practically certain that during this period he could not have been bitten by tsetse flies. He was, however, tormented by *Stomoxys* flies, which were exceedingly common at the time. Both *S. nigra* and *S. calcitrans* have been taken at Ilorin.

Treatment. The treatment usually adopted in Northern Nigeria for all cases of trypanosomiasis in horses is the administration of arsenious acid and perchloride of mercury by the mouth. This is the routine treatment adopted at the isolation camps at Zungeru and Lokoja. The doses usually employed are nine grains of the arsenic and three ounces of a 1 in 1,000 solution of the perchloride of mercury daily, divided into three doses; but I have also tried much larger doses, administered for a few days at a time only, in the hope that the sudden shock of arsenic might eliminate the trypanosomes before they had time to react to the drug, and with a view to avoiding the cumulative action on the host that has certainly sometimes proved fatal. It is doubtful whether this treatment has any beneficial results. In my experience it has not saved a single animal infected with *T. brucei*, and it is impossible to judge of its action on *T. vivax*, as this form of trypanosomiasis is as a rule not fatal, the animals recovering even when untreated. At one time it was asserted that such treatment accounted for the cure of 50 % of the cases at Zungeru. This, however, was before it was known that half the trypanosomiasis there was due to *T. vivax*, and was capable of spontaneous cure. The higher mortality recorded at Lokoja is probably accounted for by the larger percentage of cases of trypanosomiasis due to *T. brucei* which occur at that station.

Intravenous injections of antimony have met with no greater success in trypanosomiasis due to *T. brucei*. Organic arsenical compounds have been used in only a few cases, the high cost of the injections and the technical difficulties of administration standing in the way of the general use of these preparations until it is certain, not only that a cure will result, but also that immunity against future attacks will be established.

Arsenic and mercury have been used as prophylactics also, but without success.

No immunity follows an attack of trypanosomiasis, reinfections with the same or a different species of trypanosome being met with. The dwarf cow (No. 48) in my series was infected successively with *T. theileri*, *T. vivax* and *T. brucei*, and finally died of the last form of trypanosomiasis. The donkey (No. 47), after recovering from an infection with *T. vivax*, was infected by injection with *T. brucei*, and succumbed to the disease.

Treatment of any sort is, however, applicable only to Government stock and horses in the possession of Europeans. The great majority of cases, occurring in animals which are the property of natives, cannot be brought under treatment. It might be possible, nevertheless, to limit the spread of the disease by the establishment of isolation camps, and much might be effected by systematic clearing along the roads. The main caravan routes especially should be cleared, and inducement offered to the natives to farm the land on either side of the roads.

Dwarf cattle. In certain districts in which the Fulani cattle do not live a few dwarf cattle are found, which, because they occur in pagan country, are often referred to as 'pagan cattle.' In Ilorin they are kept in all the districts occupying the south-eastern corner of the province, from Ofa Ora to Ejiba on the north, and extending west along the southern boundary as far as Oke Awra. Dr. Foy, who made some interesting observations and experiments on a bull of this breed, thus described his physical characters. 'The body frame,' he says, 'was thick-set and broad and comparatively long for its height, the legs thick and short, the neck thick and short, the head short, and the horns short.'[8] The accompanying photograph of a bull and cow brought from Awtun will help to give an accurate idea of the appearance of these singular animals (Pl. III, fig. 5).

The most remarkable point about these dwarf cattle, which are bred for slaughter only and are not used for milking, is that they live in districts in which the Fulani cattle cannot exist. For this reason it has been conjectured that they have acquired a high degree of immunity to trypanosome infections. Dr. Foy, who, as already stated, experimented on a bull of this breed, concluded:—

'(1) That a certain breed of cattle found in pagan districts possess a high degree of natural immunity in that they may harbour the trypanosome in the blood and yet keep in good condition and

show no signs of the disease, nor do they die from the infection so long as their environments are favourable. These environments are a free life, with ample food, especially plenty of green grass. Confinement, poor feeding, and hard exercise, tend to make the disease manifest itself clinically.

'(2) That such domesticated cattle may act as a reservoir of infection since the blood may prove infective at such times when clinical symptoms manifest themselves, although the trypanosome may not be found on making a microscopical examination.

'(3) That the subcutaneous injection of 26 c.c. of serum obtained from the blood of one of these cattle when manifesting no clinical symptoms of the disease did not prove infective when inoculated into a calf, nor did it act as a prophylactic when the calf was exposed to natural infection subsequently.'

He adds, in another part of the same paper, 'all strains of trypanosomes used or met with in the work were of the *T. brucei* type.'

At Ilorin I was able to carry out only two experiments with these cattle, but as the subject requires careful investigation, and promises to provide results of practical importance, I should, perhaps, place them on record here. On May 11th, 1912, two of these dwarf cattle (a cow and a bull) arrived at Ilorin from Awtun. As they had treked up all the way by road, they arrived in rather poor condition, but seemed, on the whole, to have stood the unusual exertion wonderfully well. On May 13th I made a thorough examination of the blood of each without detecting any trypanosomes, and four days later injected a rat and a guinea-pig each with 1 c.c. of blood taken from the cow. Neither of these animals developed any symptoms of disease, and, although they were kept under close and constant observation for four weeks, trypanosomes were never seen in their blood. The subsequent history of the cattle is given below:—

Dwarf Bull (No. 50)

May 11th, 1912.—Arrived at Ilorin.

May 13th, 1912.—Blood examination—negative.

August 30th, 1912.—Trypanosomes (*T. vivax*) in the blood. Bull looks well. Untreated.

September 15th, 1912.—Blood examination—negative. Bull, however, looks ill.

September 19th, 1912.—Bull died of anthrax. No trypanosomes found in the blood nor in smears made from the organs after death.

Dwarf Cow (No. 48)

May 11th, 1912.—Arrived at Ilorin.

May 13th, 1912.—Blood examination—negative.

May 17th, 1912.—Blood examination—negative. Subcutaneous injections of the cow's blood made into a rat and a guinea-pig.

Rat under observation twenty-eight days, but never showed trypanosomes.

Guinea-pig under observation twenty-eight days but never showed trypanosomes.

August 30th, 1912.—Cow feverish and emaciated, no oedemas. Trypanosomes (*T. theileri* and *T. vivax*) present in the blood. Untreated.

September 7th, 1912.—Trypanosomes still present in the blood. Subcutaneous injection of two c.c of the cow's blood into a guinea-pig.

Guinea-pig under observation nineteen days. Accidentally killed. Never showed trypanosomes.

September 18th, 1912.—Blood examination—negative. One c.c. of the cow's blood injected subcutaneously into a rat.

Rat under observation twenty-two days but never showed trypanosomes.

September 27th, 1912.—Cow looks well, and her blood has been free from trypanosomes since September 15th. Two c.c. of blood from donkey (No. 45), heavily infected with *T. brucei*, injected into cow subcutaneously.

October 8th, 1912.—Trypanosomes numerous (*T. brucei*). The blood was negative up to October 5th.

November 3rd, 1912.—Cow died. Trypanosomes still present in the blood but scanty.

Both animals developed a natural infection with *T. vivax*, and apparently recovered, for they ceased to show trypanosomes in films made from the peripheral blood. The cow in addition had a concurrent infection with *T. theileri*. The cow was then successfully infected by injection of blood from a donkey heavily infected with *T. brucei*, and, although the parasites soon became scarce in her blood, she died five weeks after the injection still showing a few trypanosomes in the peripheral blood.

It would be rash to draw definite conclusions from such experiments, but so far as they go they do tend to show that this breed of cattle, like horses, possesses an immunity to *T. vivax*, inasmuch as although these parasites may appear in their blood they do not produce a rapidly fatal disease. The immunity does not appear, however, to extend to *T. brucei*. The question then arises how do these cattle live in districts where the Fulani herdsmen dare not take their cattle to graze? The dwarf cattle are generally to be found in the immediate vicinity of villages, and unlike the Fulani cattle do not wander widely over the country in search of pastures. It was thought possible, therefore, that they might thus escape the attacks of *G. submorsitans*, the species of tsetse fly whose distribu-

tion (as already pointed out) is coextensive with the areas habitually shunned by the Fulani herdsmen. In consequence, collections of flies were obtained at Odo Okeri, Eri, Oro and Oke Onio, from spots where the cattle were actually grazing. In none of these collections was *G. submorsitans* present, although both *G. palpalis* and *G. tachinoides* were taken. I have to thank Mr. T. A. G. Budgen for very kindly superintending the work of a collector who was sent with him for this purpose. I can only hope that at some future date, and under more favourable conditions, it may be possible to make a thorough investigation of this interesting subject.

In conclusion, I would like to express my thanks to Mr. E. C. Duff for affording me an opportunity of examining the dwarf breed of cattle; to Dr. C. F. Watson and Dr. W. Morrison for very kindly sending me blood-films from Lokoja; to Dr. G. R. Twomey, who relieved me at Ilorin, for following the last stages of my experiments; and to Serjt. Moore for the careful record kept by him of the cases of trypanosomiasis occurring at Zungeru.

A tabular synopsis of the cases of trypanosomiasis in domestic animals, collected in Northern Nigeria, is appended.

SYNOPSIS OF CASES OF TRYPANOSOMIASIS OF DOMESTIC ANIMALS

COLLECTED IN NORTHERN NIGERIA

No.	Place	Host	Date	Identification	Result	Remarks
1	Minna	Horse	Aug., 1910	*T. vivax* *	Recovered	Mild case, no oedema.
2	Bida	,,	Sept., 1910	*T. nanum* or *pecorum**	,,	
3	Zungeru	,,	Nov., 1910	*T. vivax* *	,,	
4	,,	,,	,,	*T. vivax* *	(?)	Died of intercurrent disease.
5	,,	,,	Jan., 1911	*T. brucei* *	Died	Ill 2 months. Latterly extremely emaciated, refusing food and water, unable to rest and constantly walking round and round in a small circle with the head bent almost down to the ground.
6	Benue R. near Umaisha	,,	Dec., 1910	*T. brucei* *	(?)	
7	Ankpa	,,	Jan., 1911	*T. vivax* *	(?)	A severe case. A large swelling formed under the throat, burst in a few days, and discharged large quantities of watery fluid.
8	Zungeru	,,	,,	*T. vivax* *	Recovered	
9	,,	,,	,,	*T. brucei* *	Died	Ill 7 weeks.
10	,,	,,	Feb., 1911	*T. vivax* *	Recovered	
11	,,	,,	,,	*T. vivax* *	,,	
12	,,	,,	Mar., 1911	*T. brucei* *	Died	Also microfilariae. Ill 5 months.
13	,,	,,	May, 1911	*T. vivax* *	Recovered	
14	,,	,,	,,	*T. vivax* *	,,	
15	,,	,,	,,	*T. vivax* *	,,	

* Identifications by Sir David Bruce.

SYNOPSIS OF CASES OF TRYPANOSOMIASIS OF DOMESTIC ANIMALS. COLLECTED IN NORTHERN NIGERIA.—*continued.*

No.	Place	Host	Date	Identification	Result	Remarks
16	Lokoja	Horse	Oct., 1911	*T. brucei* †	Died	Had recovered from a previous attack of trypanosomiasis.
17	,,	,,	,,	*T. vivax* †	Recovered	
18	,,	,,	,,	*T. brucei* †	Died	'Ill for months.'
19	,,	,,	Nov., 1911	*T. brucei* †	(?)	'Ill for mouths.'
20	,,	,,	,,	*T. vivax* †	Recovered	
21	,,	,,	,,	*T. nanum* or *pecorum*	(?)	Sold to a native.
22	,,	,,	Mar., 1912	*T. nanum* or *pecorum*	Died	
23	,,	,,	Feb., 1912	*T. vivax*	,,	'Very marked oedema of joints, legs, abdomen and testicles.'
24	Zungeru	,,	Nov., 1911	*T. brucei*	,,	
25	,,	,,	Dec., 1911	*T. vivax*	Recovered	
26	,,	,,	Jan., 1912	*T. vivax*	(?)	
27	,,	,,	,,	*T. nanum* or *pecorum*	Recovered	But see No. 33. Lachrymal and nasal discharges. Guinea-pig injected with negative result.
28	,,	,,	,,	*T. nanum* or *pecorum*	(?)	Sold to a native.
29	,,	,,	,,	*T. nanum* or *pecorum*	(?)	
30	,,	,,	Feb., 1912	*T. brucei*	(?)	

† Identifications by Dr. Blacklock, The Runcorn Research Laboratory.

Synopsis of Cases of Trypanosomiasis of Domestic Animals. Collected in Northern Nigeria.—*continued.*

No.	Place	Host	Date	Identification	Result	Remarks
31	Zungeru	Horse	Feb., 1912	*T. nanum* or *pecorum*	(?)	
32	Ilorin	,,	,,	*T. vivax*	Recovered	Also microfilariae present.
33	,,	,,	April, 1912	*T. nanum* or *pecorum* and *T. vivax*	Died	Same horse as No. 27. Ill 19 days. Very marked oedema subsiding latterly leaving the pony a mere bag of bones. Profuse diarrhoea.
34	Ilorin	,,	June, 1912	*T. vivax*	Recovered	Mild infection.
35	Offa	,,	July, 1912	*T. brucei*	Died	Pl. I, fig. 1. Microfilariae also present. Ill 30 days. Posterior nuclear forms present; oedema slight. In rat incubation 6 days, death on 26th day after injection. In guinea-pig incubation 8 days, death on 11th day after injection.
36	Ilorin	,,	Aug., 1912	*T. nanum* or *pecorum*	Recovered	Also microfilariae present.
37	Patigi	,,	,,	*T. brucei*	Died	Posterior nuclear forms present. Ill 11 days.
38	Ilorin	,,	,,	*T. brucei*	,,	Microfilariae detected in pony's blood last April. Ill 22 days. Eye symptoms present.
39	,,	,,	,,	*T. nanum* or *pecorum* and *T. brucei*	(?)	Still ill on October 19th. *T. brucei* very scanty. A very young pony.
40	,,	,,	,,	*T. vivax*	Recovered	
41	,,	,,	Sept., 1912	*T. vivax* and *T. brucei*	Died	Ill about 2 months.

No.	Place	Host	Date	Identification	Result	Remarks
42	Ilorin	Horse	Oct., 1912	*T. brucei*	Died	Pl. I, fig. 2. Possibly infected directly by Stomoxys from donkey No. 45 or No 47. Posterior nuclear forms present. Well marked oedema. Ill 2 weeks. In guinea-pig incubation 19-23 days, death 42nd day.
43	Patigi	,,	,,	*T. brucei*	,,	Ill about 3 weeks.
44	Ilorin	Donkey	Aug., 1912	*T. brucei*	,,	Pl. II, fig. 3. Posterior nuclear forms present. Slight oedema. Ill about 3 weeks. In rat incubation 10 days, still alive on 34th day. In guinea-pig incubation 10 days, died on 58th day.
45	,,	,,	Sept., 1912	*T. brucei*	,,	Pl. II, fig. 4. Ill about 8 weeks.
46	,,	,,	,,	*T. vivax*	,,	
47	,,	,,	,,	*T. vivax*	Recovered	
47A	,,	,,	Oct., 1912	*T. brucei*	Died	Injected from donkey No. 45. Died 24 days after injection. Little or no oedema.
48	,,	Dwarf cow	Aug., 1912	*T. theileri*	Recovered	Pl. III, fig. 5.
49	,,	,,	Sept., 1912	*T. vivax*	,,	
49A	,,	,,	Oct., 1912	*T. brucei*	Died	Injected from donkey No. 45. Died 37 days after injection.
50	,,	Dwarf bull	Aug., 1912	*T. vivax*	Recovered	
51	,,	Fulani cow	Sept., 1912	*T. nanum* or *pecorum*	Slaughtered	Treking South.
52	,,	,,	,,	*T. vivax*	,,	,, ,,
53	,,	,,	,,	*T. vivax*	,,	,, ,,
54	,,	Fulani bull	,,	*T. vivax*	,,	,, ,,

SYNOPSIS OF CASES OF TRYPANOSOMIASIS OF DOMESTIC ANIMALS. COLLECTED IN NORTHERN NIGERIA.—*continued*.

No.	Place	Host	Date	Identification	Result	Remarks
55	Ilorin	Fulani cow	Sept., 1912	*T. vivax*	Slaughtered	Treking South from Kano.
56	,,	Fulani bull	,,	*T. vivax*	,,	,, ,, ,,
57	,,	,,	,,	*T. vivax*	,,	,, ,, ,,
58	,,	Fulani cow	,,	*T. vivax*	,,	,, ,, ,,
59	,,	Fulani bull	,,	*T. vivax*	,,	,, ,, ,,
60	,,	,,	,,	*T. vivax*	,,	,, ,,
61	,,	,,	,,	*T. vivax*	,,	,, ,, ,,
62	,,	,,	,,	*T. vivax*	,,	,, ,, ,,
63	,,	,,	,,	*T. vivax*	,,	,, ,,
64	,,	,,	,,	*T. vivax*	,,	Treking South from Kano. Ill 10 days.
65	,,	,,	,,	*T. vivax*	,,	,, ,, ,, ,, ,,
66	,,	Fulani cow	,,	*T. nanum* or *pecorum* and *T. vivax*	Sent to Ibadan to be slaughtered	Ilorin cow.
67	,,	,,	,,	*T. vivax*	Slaughtered	Treking South from Kano.
68	,,	,,	Oct., 1912	*T. brucei*	,,	,, ,, ,, (Jebba route). Posterior nuclear forms present.
69	,,	,,	,,	*T. vivax*	,,	,, ,, from Hadeija (Patigi route).
70	,,	,,	,,	*T. vivax*	,,	,, ,, from Hausa country (Jebba route).
71	,,	,,	,,	*T. vivax*	,,	,, ,, ,, ,, ,,

SYNOPSIS OF CASES OF TRYPANOSOMIASIS OF DOMESTIC ANIMALS. COLLECTED IN NORTHERN NIGERIA.—*continued.*

No.	Place	Host	Date	Identification	Result	Remarks
72	Ilorin	Sheep	Sept., 1912	*T. vivax*	Slaughtered	
73	,,	Ram	,,	*T. brucei*	,,	Long forms 21 μ, stumpy 13-14 μ
74	,,	,,	,,	*T. vivax*	,,	Treking South from Kano.
75	,,	,,	,,	*T. vivax*	,,	,, ,, from North.
76	,,	Sheep	,,	*T. vivax*	,,	,, ,, from Kano.
77	,,	Ram	,,	*T. vivax*	,,	,, ,,
78	,,	,,	,,	*T. vivax*	,,	,, ,, ,,
79	,,	,,	,,	*T. vivax*	,,	,, ,, ,, (Patigi route).
80	,,	Sheep	Oct., 1912	*T. vivax*	,,	From Alapa, Ilorin.
81	,,	Ram	,,	*T. nanum* or *pecorum*	,,	Treking South from Kano (Jebba route).
82	,,	Goat	Sept., 1912	*T. vivax*	,,	
83	,,	,,	,,	*T. vivax*	,,	
84	,,	,,	Oct., 1912	*T. vivax*	,,	Ilorin goat.
85	,,	,,	,,	*T. vivax*	,,	,, ,,
86	,,	Dog	Sept., 1912	*T. vivax*	Died	

SUMMARY

1. Trypanosomes presenting the morphological characters of *T. brucei*, *T. vivax*, *T. nanum* or *pecorum*, and *T. theileri* have been found in Northern Nigeria in the blood of domestic animals; *T. brucei* in the horse, donkey, Fulani cattle, dwarf cattle and sheep; *T. vivax* in the horse, donkey, Fulani cattle, dwarf cattle, sheep, goat and dog; *T. nanum* or *pecorum* in the horse, Fulani cattle and sheep; and *T. theileri* in the dwarf cattle.

2. *T. vivax* is the most common form at any rate in the south-western portions of the Protectorate, being present in fifty-five out of eighty-four cases collected in Ilorin province, the Niger province, and at Lokoja.

3. Of twenty Fulani cattle treking down towards the coast from the provinces of the north, and found to be suffering from trypanosomiasis, eighteen harboured *T. vivax*.

4. In horses *T. vivax* produces a much less serious disease than *T. brucei*. Of fifteen cases infected with *T. vivax*, of which the records are complete, fourteen recovered. Of eleven cases infected with *T. brucei* none recovered.

5. The dwarf breed of cattle found in certain tsetse-haunted districts of Northern Nigeria, and credited with a natural immunity to trypanosomiasis, while apparently recovering from infections with *T. vivax*, succumb to *T. brucei*.

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EXPLANATION OF PLATE I

Fig. 1. Horse (No. 35). Trypanosomiasis (*T. brucei*). Ilorin, July, 1912.

Fig. 2. Horse (No. 42). Trypanosomiasis (*T. brucei*). Ilorin, October, 1912.

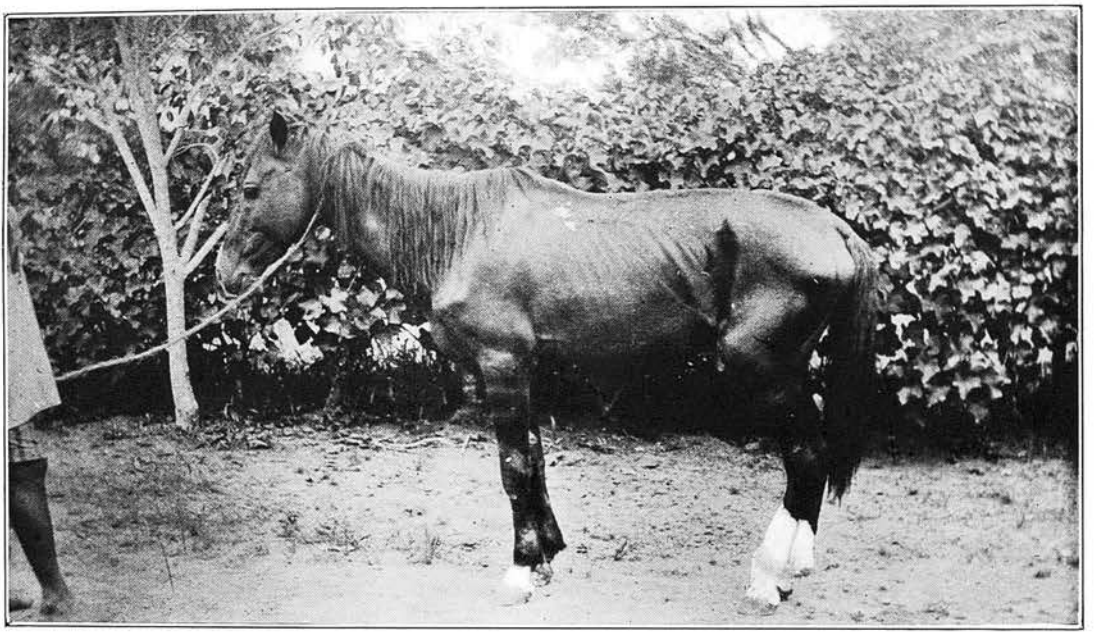


FIG. 1

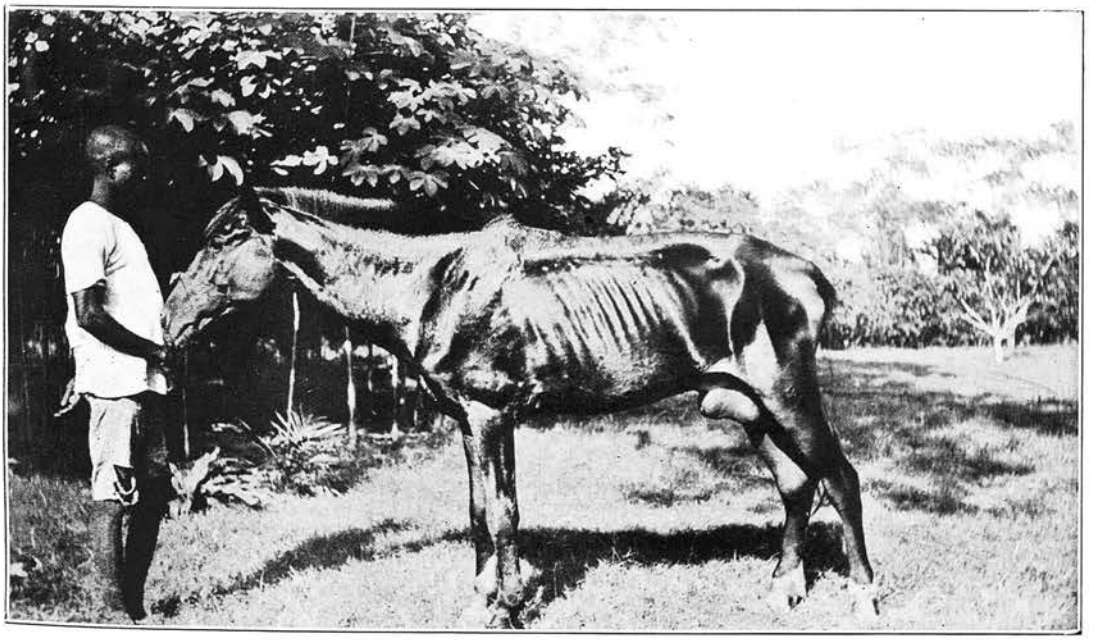


FIG. 2

C. Tinling & Co., Ltd., Imp.

EXPLANATION OF PLATE II

Fig. 3. Donkey (No. 44). Trypanosomiasis (*T. brucei*). Ilorin, August, 1912.

Fig. 4. Donkey (No. 45). Trypanosomiasis (*T. brucei*). Ilorin, September, 1912.

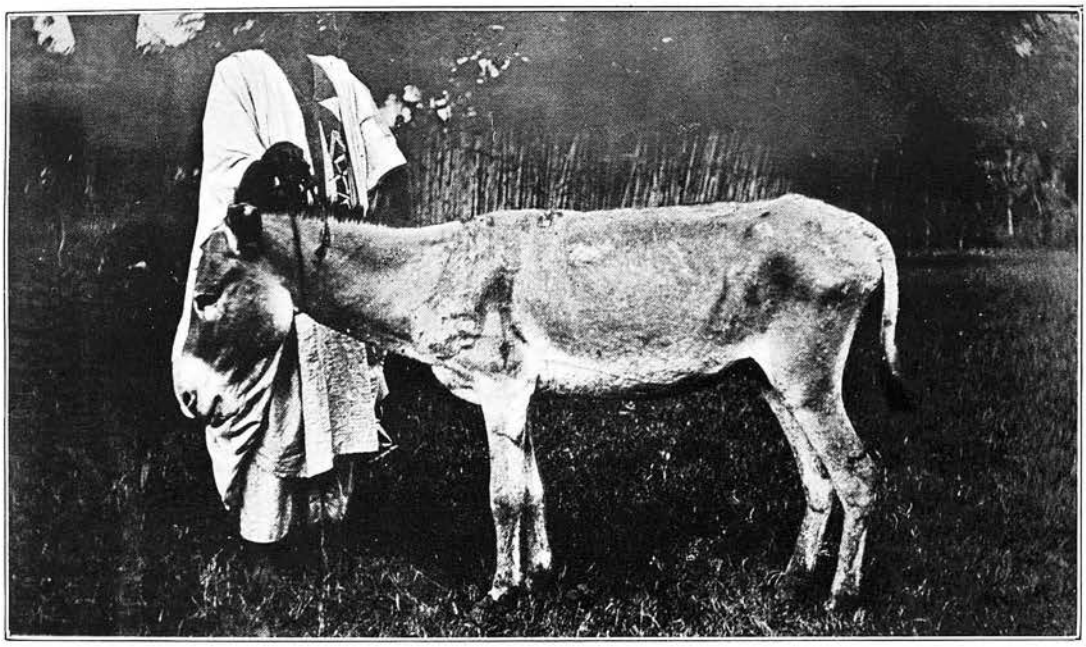


FIG. 3

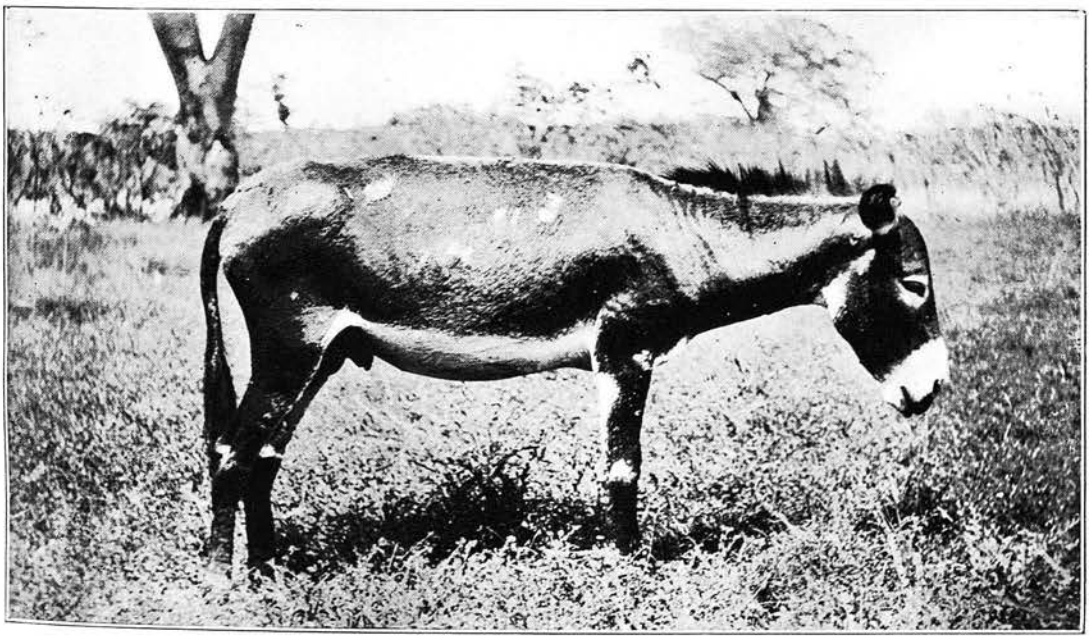


FIG. 4

C. Tinling & Co., Ltd., Imp.

EXPLANATION OF PLATE III

Fig. 5. Dwarf cattle. Ilorin, May, 1912.

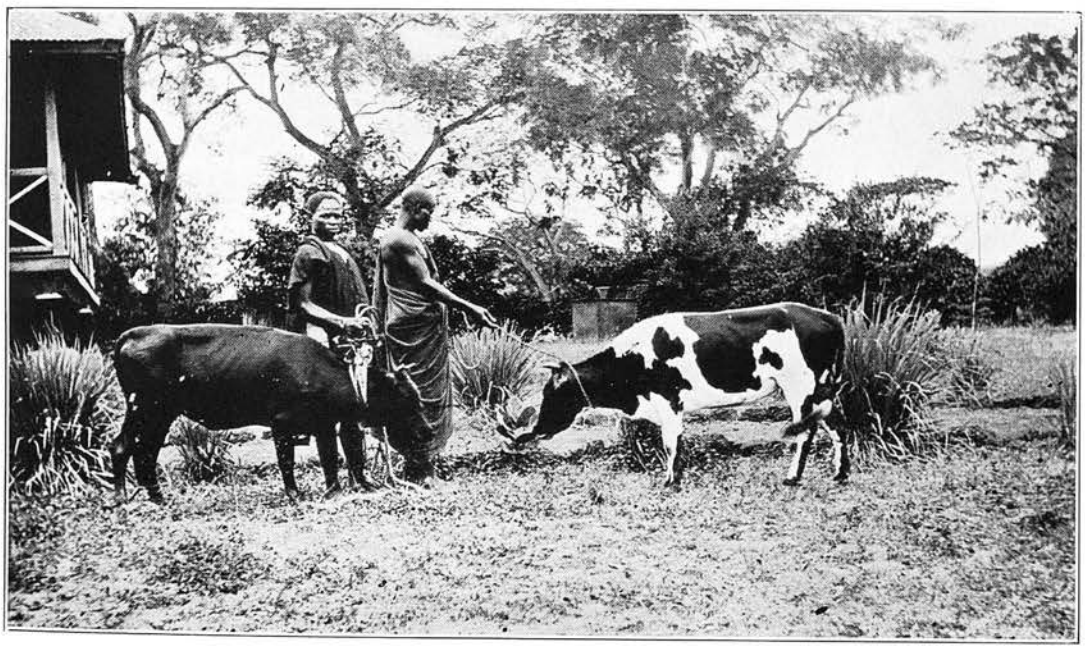
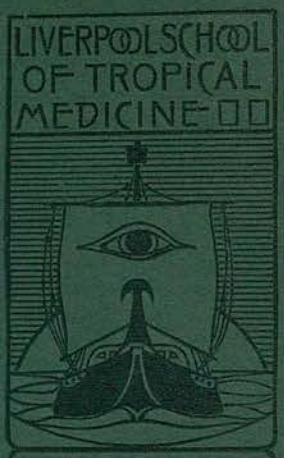


FIG. 5

C. Tinling & Co., Ltd., Imp.



ANNALS

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On the Morphology of the Trypanosome (*T. nigeriense*, n.sp.) from a Case of Sleeping Sickness from Eket, Southern Nigeria

J. W. SCOTT MACFIE, M.A., B.Sc.

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ON THE MORPHOLOGY OF THE TRYPANOSOME (*T. NIGERIENSE*, N. SP.) FROM A CASE OF SLEEPING SICKNESS FROM EKET, SOUTHERN NIGERIA

BY

J. W. SCOTT MACFIE, M.A., B.Sc.

MEDICAL RESEARCH INSTITUTE, LAGOS, SOUTHERN NIGERIA

(*Received for publication* 25 *July*, 1913)

Early in 1912 attention was drawn to the presence of a disease resembling sleeping sickness in the Eket district of Southern Nigeria. Mr. S. A. Bill, of the Qua Ibo Mission, reported in February that a girl at a village called Ikot Offiong appeared to be suffering from trypanosomiasis. A medical officer (Dr. R. W. Gray) was accordingly despatched to the district to investigate this case, and to enquire into the presence of sleeping sickness in the neighbourhood of Eket. In May he was able to report that he had discovered two cases; and, as on further investigation the disease was found to be widespread, an isolation camp was established near Ikorobo. Up to the end of April, 1913, a total of 167 cases of sleeping sickness had been identified.

Sporadic cases of sleeping sickness are known to occur in many parts of Nigeria, and in certain districts the disease appears to be endemic. The writer has seen cases at Baro and Kateri in Northern Nigeria, but although trypanosomes were present in the peripheral blood of these patients, they did not appear to be ill. In Kabba province of Northern Nigeria it is said to be endemic, and along the course of the Garara river, where the writer had an opportunity of investigating the subject in 1910, it was well known, but apparently uncommon. Sleeping sickness does not seem to be of a virulent type in Nigeria, and it does not at present occur in epidemic form. To account for this fact it has been supposed that the disease must have existed for a great number of years, and that the natives must have acquired a relative immunity. Some such

explanation is necessary since sporadic cases have been identified in so many places, and the local conditions over so much of the country are so favourable for the spread of the disease.

Sleeping sickness as it occurs at Eket, however, presents some special features. It was thought, therefore, that a study of the trypanosome producing the disease might be of interest. For this purpose Dr. Foran, the medical officer in charge of the sleeping sickness investigations, very kindly sent the writer a guinea-pig which had been inoculated from one of his cases. On examination the trypanosome was found to possess some unusual morphological features which are, I think, of sufficient interest to place on record, in view of the peculiar clinical symptoms with which they are associated.

SLEEPING SICKNESS IN EKET DISTRICT

I am indebted to the official reports of Dr. P. F. Foran for the following account of the disease as it occurs at Eket.

Sleeping sickness has, apparently, been known to the natives of the district for a very long time. It has, indeed, existed beyond the memory of the oldest inhabitants. They state, however, that it has become more common lately, and this they attribute to the fact that the present generation is inclined to ignore certain old native laws that forbade an infected person to associate with his fellows. Two phases of the disease are recognised. The early phase, characterised by enlargement of the glands of the neck, is known as *nsipiton;* and the later phase, in which lethargy appears, is called *odongo-idap.** Cases in the former stage are common, but those that have advanced to the latter stage are comparatively rare. The disease appears to be mild, although a number of deaths are reported to occur annually. In September, 1912, Dr. Foran wrote, 'I have only heard of four deaths from sleeping sickness, yet

* The writer, in 1910, found that a similar distinction was observed by the natives of Northern Nigeria. The stage of glandular enlargement they termed *chiwan wiya* (neck sickness), and the stage of lethargy *chiwan berichi* (sleep sickness). Sleeping Sickness Bureau Bulletin, No. 27, p. 236.

nsipiton [the early, glandular, phase of the disease] is very common.' The duration is a matter of years. In the cases collected, symptoms had been present for from one to five years. The great majority of the patients are children or young adults, their ages ranging from about 6 to 18 years. The sexes are about equally affected.

The symptoms are as follows: As a rule the general health is good, there is no marked anaemia, and wasting is present in only a few cases. Irregular fever occurs, especially in the afternoon and evening, and is accompanied by headache and malaise, and terminates in sweating. The tongue is furred. The spleen is almost always enlarged. The expression of some patients is dull and vacant; and there may be oedema or drooping of the eyelids. Muscular tremors occasionally occur. Weakness or lassitude is always more or less well marked. Skin rashes—urticaria and erythema—are present in many cases; but they are also common in uninfected natives. Impotence and amenorrhoea are said to accompany the disease. Enlargement of the cervical and axillary glands is the most constant sign. The glands are freely movable, soft, and elastic; and on microscopical examination are found to contain trypanosomes. Enlarged glands in other sites, such as the groin, do not contain the parasites. On excision, the glands are found to be pale pink in colour. They do not contain pus, and it is noteworthy that those showing signs of induration are found to be free from trypanosomes. The natives believe that the disease is curable by the excision of the glands, an operation which their 'doctors' readily perform. Numerous healthy individuals bearing scars on their necks are to be met with who have, it is affirmed, been cured in this manner.* According to the natives the glandular phase of the disease (*nsipiton*) lasts for about four years before lethargic symptoms develop. From the fact that somnolence is not a common symptom, most of the patients appearing to be in the early stages, it is possible that many of them recover before this stage is reached.

Trypanosomes are found in the gland juice of practically every

* Compare the note on the excision of cervical glands by a native doctor in Kabba province, Northern Nigeria. Dr. W. Morrison. British Medical Journal, June 8th, 1912.

case, but, up to the present, they have not been detected in the peripheral blood. In a few cases presenting the appearances of advanced sleeping sickness trypanosomes have not been found at all. In them the glands are but little enlarged, and, as noted above, indurated glands are generally free from parasites.

The country around Eket is an undulating plateau covered with dense bush, and intersected by numerous waterways. It includes a good deal of swampy land. The district is densely populated. The towns consist of scattered compounds standing some distance apart, and closely surrounded by farms and banana trees. They are usually very dirty, and some cattle, and a good many pigs, goats, and fowls are kept in them. The towns are generally some distance away from the water-side. Their water supply is obtained at two or three spots, which are closely surrounded by bush and trees, and approached along a narrow shaded path. The children and young adults are the water-carriers, and Dr. Foran considers that this accounts for the majority of the cases of sleeping sickness occurring in young people. Tsetse flies are prevalent all over the district. Dr. J. J. Simpson records *G. palpalis* and *G. caliginea* from Eket in his map of Southern Nigeria, showing the distribution of the genus *Glossina;** but, according to Dr. Foran, *G. tachinoides* is also a common species, at any rate, during the months of September and October.† He has observed that the tsetse flies 'appear to follow pigs about more than any other animal, and it is generally easier to catch the flies where these animals are than at the water.'

THE STRAIN OF TRYPANSOME

The trypanosomes used in this investigation were found in the blood of a guinea-pig kindly sent to the writer from Eket by Dr. P. F. Foran. The guinea-pig had been inoculated with cerebro-spinal fluid from a case of sleeping sickness on March 5th, 1913, but the incubation period is not known. The animal reached Lagos on May 8th, 1913, and was found to be infected with

* Bulletin of Entomological Research, Vol. III, part 2, 1912.

† If true, this is a remarkable fact as *G. tachinoides* does not occur so near to the coast elsewhere in Southern Nigeria. It is generally only met with inland, beyond the forest zone.

trypanosomes. It appeared to be well, and, at the time of writing (June 11th, 1913), is still in good health, although it is now 97 days since the date of inoculation. The trypanosomes have never been very numerous in the blood. Their numbers have not sensibly increased during the last month. It is, therefore, impossible to tell to what stage the disease has at present advanced.

The red blood corpuscles of the host exhibit well marked polychromasia and basophilia. In many of them, too, irregularly shaped fragments are to be seen, which stain red with Gimesa's solution.

MORPHOLOGY OF THE TRYPANOSOME FROM EKET

In this paper the flagellar end of the trypanosome will be termed anterior, and the non-flagellar end posterior.

A—*Living, unstained.*

The trypanosome from Eket in the fresh condition, as seen in a drop of blood from an infected guinea-pig, appears as an elongated tapering body of almost homogeneous consistency. The micronucleus can sometimes be distinguished as a small refractile body. The movements are active. Some individuals, which appear to be stouter than the others, are relatively sluggish, and do not move actively about the field. Others, which are long and slender, vibrate exceedingly actively, and also move rapidly across the field of the microscope. The translatory movement seems to be spasmodic, the trypanosome suddenly gliding across the field after having been more or less stationary, but vibrating vigorously all the time, for some moments previously. As a rule, the flagellar end moves forwards in the translatory movements. The non-flagellar end appears to be blunt, the flagellar attenuated. The undulating membrane is conspicuous.

In blood that has been shed for some little time changes may be observed taking place in the form of some of the trypanosomes. The writer has watched these changes on several occasions. They always occurred in long slender parasites, and were accompanied by a diminution of the activity of the trypanosome. The non-flagellar end of the trypanosome appears to round off, and eventually the

body becomes almost spherical. These atypical parasites are occasionally met with in stained blood films. One is illustrated (Plate XXXIII, fig. 22).

B—*Fixed and stained.*

Method of fixation and staining.—The blood films were fixed either with absolute alcohol or with osmic acid. The forms of the trypanosome were found to be the same in either case, but with osmic acid fixation the appearance of the cytoplasm was different if the films were stained very deeply. All the films were stained with Giemsa's solution (about 30-35 drops in 10 c.c. of water); those fixed with alcohol being stained for one hour, and those fixed with osmic acid for from ten to fifteen minutes.

The longest trypanosome measured, up to the present, is 32μ, and the shortest 8μ.

Breadth.—The breadth of the trypanosomes at the widest part varies from 1μ to 2·5μ.

Shape.—The trypanosome when stained is seen to be polymorphic. Long slender forms, short stumpy forms, and intermediate forms are always present; but their relative proportions vary considerably from day to day. The trypanosomes have never been very numerous, and, as a rule, the long slender forms have predominated. The long slender forms are of two types. In the one type the blepharoplast is terminal, and the nucleus elongated (Plate XXXIII, fig. 1). In some specimens the nucleus appears to have a clear area in the middle which gives it the appearance of being made up of two pieces, and the body anterior to it is often attenuated. In the other type the blepharoplast is situated about 1μ from the posterior extremity, which is prolonged beyond it into a blunt snout (Plate XXXIII, figs. 2, 3). The intermediate forms are very variable in size and general appearance. Some are pointed at both ends, others have a blunt posterior extremity, which is occasionally rounded off like the head of a tadpole. The blepharoplast is either terminal or sub-terminal. The body ends anteriorly in a very short 'free' flagellum. The stumpy forms are exceedingly short in some instances (Plate XXXIII, figs. 11, 12), and may measure as little as 8μ. They are relatively very broad,

measuring about 2μ. At the anterior end there is a very short 'free' flagellum. The membrane is not broad. The posterior end is quite blunt, and the beginning of the flagellum may form the posterior border of the parasite. The blepharoplast, as a rule, lies at the edge of the trypanosome a little way in front of the posterior end of the body, and on the opposite edge the folds of the membrane pass forwards. The nucleus is large, and is sometimes placed at the extreme anterior end (Plate XXXIII, fig. 13).

From time to time, in films prepared in the usual way, there appear in the blood trypanosomes which have the flagellum free in its entire length (Plate XXXIII, figs. 14, 15). They have not been observed in unstained blood-films, and, indeed, they would be very difficult to make out in fresh preparations. It is, therefore, possible that they may be artefacts; but it is curious that they have never been seen before by the writer in any other animal, although he invariably prepares his blood films in exactly the same way. These forms are long slender trypanosomes with the blepharoplast situated some way from the extremity. The anterior end is drawn out into a filamentous termination. The body measures about 22μ, and the flagellum 31μ.

Contents of the Cell.—In well stained specimens the protoplasm is homogeneous in structure, neither granules nor vacuoles being present. In films that have been over-stained, especially if they have been fixed with osmic acid, both granules and vacuoles appear (Plate XXXIII, figs. 20, 21). The granules are coarse, and occur on both sides of the nucleus. Sometimes they almost fill up the body of the trypanosome.

The nucleus.—The nucleus is oval, and about 2·5μ in length. The nucleus of the long slender trypanosomes with a terminal blepharoplast has already been referred to. In the stumpy forms the nucleus is rounded, and is generally surrounded on all sides by a zone of protoplasm. The nucleus is situated near the centre of the body in the majority of the trypanosomes, but in a few, and these are always stumpy forms, it is placed anteriorly—that is, at the flagellar end (Plate XXXIII, fig. 13). This is an interesting feature, as it suggests that the position of the nucleus in the stumpy forms is an index of the virulence of the trypanosome. In the typical *T. gambiense* it is central, in the more virulent

T. rhodesiense it is sometimes posterior, and in this less virulent strain of Nigeria it is anterior.

Blepharoplast.—The position of the blepharoplast has already been mentioned. It is well marked, round, and deeply staining.

Undulating membrane.—In the long slender forms the undulating membrane is ample, and is thrown into a number of folds. In some it is apparently absent, and the flagellum is, in consequence, free in its whole length. The membrane is less folded in the stumpy and intermediate forms. In carefully prepared specimens the membrane can be followed almost to the extremity of the flagellum, even in the slender forms which at first sight appear to have a long 'free' flagellum (Plate XXXIII, fig. 4).

Flagellum.—The flagellum stains deeply. It is well marked in all forms, and in all forms the terminal portion is free—that is, projects beyond the protoplasm of the cell and the undulating membrane. As already stated, in some forms of long slender parasites, in stained preparations, the flagellum appears to be free in its entire length.

Curve of Measurements:—

In their paper entitled 'Further measurements of *Trypanosoma rhodesiense* and *T. gambiense*'[1] Prof. Stephens and Dr. Fantham have pointed out that in measuring trypanosomes it is advisable to confine observations to a single animal, as it is possible that the size of the parasite may vary in different hosts. They also recommend that the trypanosomes should be measured on ten consecutive days so as to obviate the errors due to the daily variation in the numbers of short and long forms. They suggest that 100 trypanosomes should be measured on each of the first ten days of the infection in a white rat. It has been impossible for me to follow this plan exactly because no white rats have been available, and, in the few animals that have been infected by sub-inoculation, the parasites have been extremely rare at the commencement of the disease, and have, indeed, been apparently absent altogether from the peripheral blood for several days at a time. I have, therefore, measured 100 trypanosomes on each of the ten days from the 96th day to the 105th day after inoculation in the original guinea-pig sent to me from Eket.

With this exception, I have endeavoured to follow the procedure of Stephens and Fantham as closely as possible, so that my figures might be comparable with those given by these authors for *T. rhodesiense* and *T. gambiense*.

Thin blood smears, dried in the air, were fixed in absolute alcohol and stained with Giemsa's solution. The trypanosomes were then drawn with a camera lucida, and the length of a line drawn through the middle of each was measured by the 'tangent line' method. This method is, I believe, the most accurate that has as yet been described.

In the following tables the results of these measurements are given in detail, so that a closer analysis than mere averages would permit may be made by anyone who should wish to do so. A summary of the measurements is added, and a table showing the distribution into the stumpy, intermediate, and long form groups of Bruce.

Table 3 shows the great variation in the number of intermediate and long forms of trypanosome on particular days. The variation is not, however, so great in the percentage of stumpy forms, ranging only between 66 % and 36 %. On comparing this table with that given by Stephens and Fantham, it will be seen that there is a lesser degree of variation in the figures for all three groups, and in particular the number of the intermediate forms is strikingly less. Intermediate forms, that is trypanosomes measuring from 22μ to 24μ, were indeed comparatively scarce, although actually they were among the most common lengths of parasite. The relatively slight degree of variation may have been due to the period to which the disease had advanced in this animal. The figures given by Stephens and Fantham referred to a rat during the first ten days of the disease, those dealt with in this paper were from a guinea-pig between the 96th and the 105th day after inoculation.

TABLE I.—Distribution in respect to length of 1,000 non-dividing individuals of trypanosome from a single guinea-pig

Day after inoculation		In Microns																											Averages	
		8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	Of each 20	Of each 100
96th day.	1	...	...	...	1	...	2	...	2	5	...	1	...	2	...	1	1	2	1	1	...	...	...	1	...	...	...	...	18·95	
	2	...	...	...	...	1	...	...	1	4	1	1	1	...	5	1	1	...	2	...	...	1	...	1	...	...	...	...	20·15	
	3	...	...	...	...	...	1	...	1	2	2	2	2	...	2	2	2	2	2	...	...	...	...	...	...	...	...	...	19·90	19·28
	4	...	...	1	...	1	...	2	2	2	1	1	1	2	1	2	1	1	2	...	...	...	...	...	...	...	...	...	18·40	
	5	...	...	1	...	1	1	2	1	1	...	...	2	2	2	2	1	2	1	1	...	...	...	...	...	...	...	...	19·00	
97th day.	6	...	...	...	...	...	...	1	...	...	...	...	1	2	2	4	2	2	2	1	...	1	...	...	...	...	...	...	22·75	
	7	...	...	...	...	...	...	1	...	1	1	2	1	...	2	3	2	1	...	2	1	2	1	...	...	...	...	...	22·20	
	8	...	...	...	...	1	...	2	...	...	1	2	1	1	1	2	3	1	1	1	1	...	2	...	...	...	...	...	21·25	22·20
	9	...	...	...	...	...	...	...	2	...	1	...	...	1	3	5	2	...	2	2	...	...	1	...	...	...	...	1	22·55	
	10	...	...	...	...	...	...	...	1	...	1	1	2	...	1	**2**	4	2	5	...	1	...	...	...	...	...	...	...	22·25	
98th day.	11	...	...	...	...	...	...	...	2	1	...	1	1	2	1	2	...	2	3	2	2	...	...	1	...	...	...	...	22·35	
	12	...	...	...	...	...	...	...	1	1	...	4	2	...	...	1	4	3	1	...	...	1	2	...	...	...	...	...	21·90	
	13	...	...	...	...	1	...	1	2	1	1	1	4	1	3	...	3	1	...	...	...	1	...	...	...	...	...	...	19·35	20·45
	14	1	...	...	1	...	...	...	2	2	4	...	2	2	1	...	...	...	1	1	2	...	...	1	...	...	...	...	19·15	
	15	...	1	...	...	...	1	...	2	1	2	3	1	...	1	2	1	3	...	...	1	1	...	...	...	...	...	...	19·50	
99th day.	16	...	...	...	...	1	1	1	1	...	...	2	...	...	...	...	...	2	2	1	1	3	2	2	1	...	...	...	23·70	
	17	...	...	...	...	1	...	...	...	...	2	1	2	...	...	1	2	1	...	4	...	3	2	...	...	1	...	...	23·60	
	18	...	...	...	1	...	...	...	...	...	2	5	1	1	3	2	...	1	1	...	1	...	...	2	...	...	...	...	20·85	22·99
	19	...	...	...	...	...	...	...	...	3	...	...	1	2	1	1	1	2	3	...	2	...	3	...	1	...	...	...	23·40	
	20	...	...	...	...	...	...	...	...	1	3	...	...	2	1	...	3	2	3	...	2	...	...	...	2	1	...	...	23·40	
100th day.	21	...	...	...	...	1	...	...	...	...	1	2	6	3	2	3	1	...	...	1	...	...	...	...	...	...	...	...	19·80	
	22	...	...	...	...	...	...	...	1	2	3	3	3	1	2	1	1	2	...	1	...	...	...	...	...	...	...	...	19·50	
	23	...	...	1	...	1	1	...	1	...	1	...	1	3	1	2	1	1	1	4	...	...	...	1	...	...	...	...	20·85	20·31
	24	...	...	...	1	...	...	...	1	2	1	2	2	1	1	5	1	...	...	...	2	...	1	...	...	...	...	...	20·30	
	25	...	...	...	...	...	...	2	2	...	...	3	1	2	1	1	...	2	2	1	2	...	...	1	...	...	...	...	21·10	

TABLE I.—*Continued*

Day after inoculation	In Microns																													Averages	
	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	Of each 20	Of each 100
101st day. 26	...	...	...	...	...	...	...	...	...	2	2	1	1	3	3	2	3	...	...	2	...	1	...	...	...	...	...	21·95	
27	...	...	...	...	...	...	1	...	...	...	4	3	1	3	2	...	1	2	1	...	...	2	...	...	...	...	...	21·40	
28	...	...	...	...	1	1	1	1	...	2	...	2	3	...	1	...	1	1	3	1	2	...	...	...	...	...	...	20·90	21·14
29	...	...	...	...	1	...	1	1	...	2	2	2	1	...	1	1	3	2	2	1	...	...	...	...	...	...	...	20·75	
30	...	...	...	...	...	1	1	...	1	...	2	2	1	4	2	4	...	...	1	...	...	...	...	1	...	...	...	20·70	
102nd day. 31	...	...	...	...	...	...	...	1	2	2	...	2	...	...	1	1	1	2	3	2	1	1	...	1	...	...	...	22·90	
32	...	...	...	...	...	...	...	2	...	3	...	2	2	2	...	1	2	2	1	1	...	1	...	1	...	...	...	21·75	
33	...	...	...	...	...	...	...	2	3	3	1	...	...	1	...	...	4	2	...	2	...	...	1	1	...	...	...	21·45	22·96
34	...	...	...	...	...	...	...	1	...	1	...	2	2	1	...	...	1	1	4	1	...	1	...	3	2	...	...	24·85	
35	...	...	...	...	...	...	...	...	2	...	1	1	...	2	2	3	...	1	1	1	5	...	...	...	...	...	1	23·85	
103rd day. 36	...	...	...	...	...	...	...	1	3	...	3	1	4	1	...	1	...	2	1	2	...	...	...	1	...	...	...	21·05	
37	...	...	...	1	...	...	...	1	1	1	1	2	2	3	...	1	1	1	3	...	1	...	1	...	...	...	...	21·30	
38	...	...	...	...	...	...	...	...	1	1	2	5	...	4	1	...	...	3	1	...	...	...	1	1	...	...	...	21·60	20·80
39	...	...	...	...	...	...	2	1	1	2	...	1	1	4	...	2	1	...	...	2	1	1	1	...	...	...	...	21·35	
40	...	...	...	1	1	...	...	2	2	2	1	3	2	3	1	...	...	1	...	...	...	...	1	...	...	...	...	18·70	
104th day. 41	...	1	...	...	...	...	...	1	1	2	2	2	2	...	...	2	1	...	2	1	1	1	1	...	...	...	...	21·20	
42	...	...	...	1	...	...	1	...	...	2	1	1	1	1	2	1	...	3	2	2	1	...	...	...	...	1	...	22·30	
43	...	...	...	1	1	...	...	...	2	1	1	1	1	2	...	1	2	...	1	2	...	1	1	...	2	...	...	22·30	22·82
44	...	...	...	...	...	...	...	...	...	1	1	1	1	...	1	1	...	4	1	2	4	2	...	1	...	...	...	25·00	
45	...	...	...	...	...	1	1	1	...	1	1	...	1	4	...	...	1	...	2	2	...	1	1	2	...	...	1	23·30	
105th day. 46	...	...	...	...	...	...	...	1	1	3	1	2	2	...	2	3	...	1	...	3	1	...	...	...	...	...	...	21·25	
47	...	...	...	...	...	...	...	1	...	2	1	3	3	2	1	1	...	1	2	...	1	...	...	...	1	1	...	22·05	
48	...	...	...	...	1	...	...	...	...	1	4	3	1	2	...	2	2	...	1	...	1	1	...	1	...	...	...	21·40	21·91
49	...	...	...	...	...	...	...	...	1	2	2	1	1	4	...	...	1	2	1	...	...	3	2	...	...	...	...	22·80	
50	...	...	...	...	...	...	1	...	1	...	4	3	1	2	...	...	1	1	1	2	...	1	1	...	1	...	...	22·05	
Totals	1	2	3	8	14	10	21	42	51	64	74	84	64	85	65	64	61	67	58	48	31	32	21	17	8	2	3	Average length of 1,000 trypanosomes is 21·486μ	

TABLE II.—Summary of measurements (in microns) of the lengths of 1,000 individuals of the trypanosome from Eket, Southern Nigeria, from a single guinea-pig.

	Maximum	Minimum	Averages of each 100	Averages of each 20	Range of averages of each 20
1	30	11		18·95	
2	30	12		20·15	
96th day. 3	25	13	19·28	19·90	1·75
4	25	10		18·40	
5	26	10		19·00	
6	29	14		22·75	
7	29	14		22·20	
97th day. 8	29	12	22·20	21·25	1·50
9	34	15		22·55	
10	27	15		22·25	
11	30	15		22·35	
12	29	15		21·90	
98th day. 13	28	12	20·45	19·35	3·20
14	30	8		19·15	
15	28	9		19·50	
16	31	12		23·70	
17	32	12		23·60	
99th day. 18	30	11	22·99	20·85	2·85
19	31	16		23·40	
20	32	16		23·40	
21	26	12		19·80	
22	26	15		19·50	
100th day. 23	30	10	20·31	20·85	1·60
24	29	11		20·30	
25	30	14		21·10	

TABLE II.—*Continued*

		Maximum	Minimum	Averages of each 100	Averages of each 20	Range of averages of each 20
	26	29	17		21·95	
	27	29	14		21·40	
101st day	28	28	12	21·14	20·90	1·25
	29	27	12		20·75	
	30	31	13		20·70	
	31	31	15		22 90	
	32	31	15		21·75	
102nd day.	33	31	15	22·96	21·45	3·40
	34	32	15		24·85	
	35	34	16		23·85	
	36	31	15		21·05	
	37	30	11		21·30	
103rd day.	38	31	16	20·80	21·60	2·90
	39	30	14		21·35	
	40	30	11		18·70	
	41	30	9		21·20	
	42	33	11		22·30	
104th day.	43	32	11	22·82	22·30	3·80
	44	31	17		25·00	
	45	34	13		23·30	
	46	28	15		21·25	
	47	33	15		22·05	
105th day.	48	31	12	21·91	21·40	1·55
	49	30	16		22·80	
	50	32	14		22·05	
					Range=25·00−18·40=6·60	

TABLE III.—The trypanosome from Eket, Southern Nigeria, arranged in the three groups—(*a*) 21μ and under; (*b*) 22-24μ; (*c*) 25μ and over.

Day	96	97	98	99	100	101	102	103	104	105	Totals
Stumpy 8-21μ	66	36	58	39	62	54	41	67	43	57	523
Intermediate 22-24μ	21	35	22	18	21	24	16	8	12	13	190
Long 25-34μ	13	29	20	43	17	22	43	25	45	30	287
	100	100	100	100	100	100	100	100	100	100	1000

TABLE IV.—Comparison of the trypanosome from Eket, Southern Nigeria, and *T. gambiense* (as given by Stephens and Fantham).

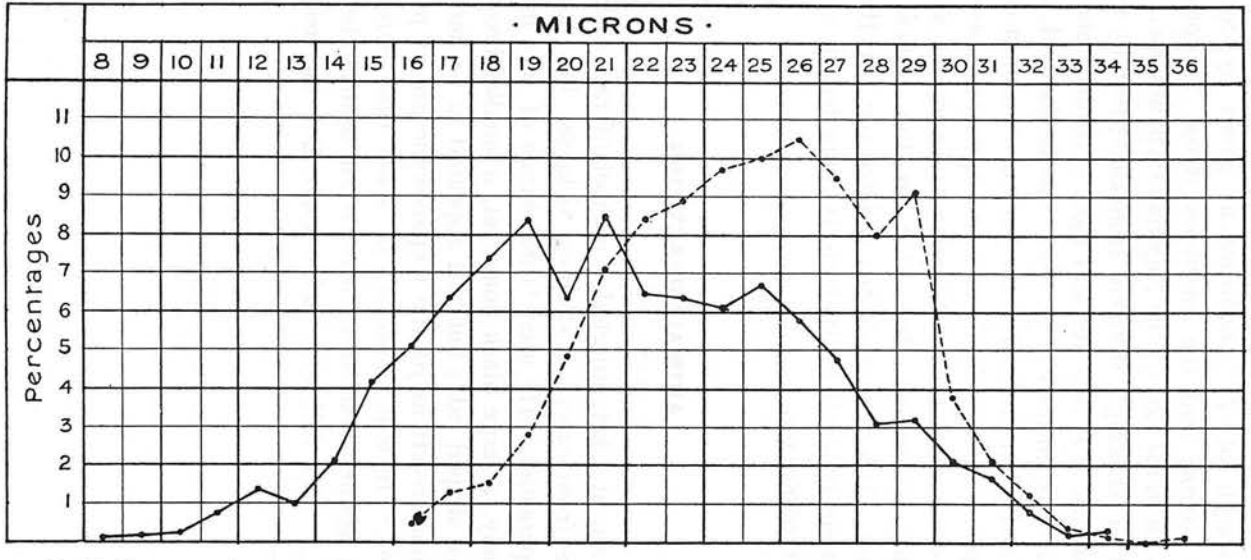
	Average	Maximum	Minimum	Under 22μ	22-24μ	25μ and over
T. gambiense	24·87μ	36·0μ	16·0μ	18·2 %	27·0 %	54·8 %
T. nigeriense	21·486μ	34·0μ	8·0μ	52·3 %	19·0 %	28·7 %

In Chart I a curve is given representing the distribution by percentages in respect to length of 1,000 non-dividing specimens of the trypanosome from Nigeria from a single guinea-pig. The curve given by Stephens and Fantham for *T. gambiense* is added on the chart to facilitate comparison.

The main object of the present investigation was to compare the measurements of the trypanosome from Southern Nigeria with those of a typical strain of *T. gambiense*. The figures have, therefore, been recorded in a manner that will permit of their being compared in detail with those given in what is, I believe, the latest exhaustive study of *T. gambiense*, that is, the paper by Stephens and Fantham already referred to repeatedly.

It will be observed that the trypanosome from Southern Nigeria is somewhat shorter than *T. gambiense;* the average length of

CHART No. I



Graphical representation of the distribution of the lengths of 1,000 trypanosomes from a guinea-pig infected at Eket (continuous line), and 1,000 *T. gambiense* from one rat (dotted line) according to Stephens and Fantham.

1,000 individuals being 21·486μ, as contrasted with 24·867μ. The maximum length of *T. gambiense* as given by Stephens and Fantham is 36·0μ, and the minimum 16·0μ, as compared with 34·0μ and 8·0μ in the case of the Nigerian trypanosome. The curve, too (Chart I), is strikingly different from that given by these authors for *T. gambiense*. It is lower and more extended, and reaches its main peak at 21μ, instead of at 26μ. At the far end, representing the exceptionally long parasites, the two curves almost coincide, but from this point backwards there is no agreement, and when the small forms are reached, the Nigerian trypanosome shows a very much higher percentage than that in the typical strain of *T. gambiense*. It has been already pointed out that the occurrence of these extremely small individuals is characteristic of this strain of human trypanosome.

ANIMAL REACTIONS

Dr. Foran, at Eket, injected a number of rats with blood and gland juice from cases of sleeping sickness, but none of them developed parasites. He used 'three kinds of rats in the experiments, namely, a large black house rat, a smaller brown bush rat, and a brown striped rat'; but 'all appeared to be immune.'

The animal reactions of the trypanosome are at present under investigation. It will, however, be some time before anything definite can be said about them, as the strain seems to be but slightly pathogenic. The following table embodies the results so far obtained:—

Animal	Days since inoculation, up to July 8	Day on which trypanosomes were first detected	Remarks
Guinea-pig 1	124	?	Alive and well. Trypanosomes numerous. The original animal from Eket.
,, 2	56	...	Has never shown trypanosomes. Alive and well.
,, 16	15	...	Died on 15th day. Never showed trypanosomes. Tissues nil.
,, 17	34	...	Has never shown trypanosomes. Alive and well.
,, 22	24	...	Has never shown trypanosomes. Alive and well.
,, 23	24	...	Has never shown trypanosomes. Alive and well.
Mouse 7	28	...	Died on 28th day. Never showed trypanosomes. Tissues nil.
,, 24	16	4	Trypanosomes always very scanty and sometimes absent. Alive and well.
,, 26	10	6	Trypanosomes scanty. Accidentally killed on 10th day.
,, 31	10	8	Trypanosomes very scanty. Alive and well.
,, 32	6	...	Trypanosomes not yet seen. Alive and well.
,, 34	6	...	Trypanosomes not yet seen. Alive and well.
,, 35	6	...	Trypanosomes not yet seen. Alive and well.
Goat 11	34	...	Has never shown trypanosomes. Alive and well.
,, 12	34	...	Has never shown trypanosomes. Alive and well.
Dog 13	34	...	Has never shown trypanosomes. Alive and well.
,, 14	34	...	Has never shown trypanosomes. Alive and well.
Monkey 21	22	10	Trypanosomes always scanty and sometimes absent. Alive and well.

SUMMARY AND CONCLUSIONS

The trypanosome, of which a preliminary account is given in this paper, seems to differ in several respects from a typical strain of *T. gambiense*. In man it produces a form of sleeping sickness that is relatively mild, occurs most commonly in young people, and in which the trypanosomes are, apparently, either absent from the peripheral blood altogether, or present in such small numbers that hitherto they have not been detected. To the smaller laboratory animals the strain seems to be but slightly pathogenic. The morphology of the trypanosome as it appears in the blood of a guinea-pig shows some peculiar features. The trypanosome is smaller than *T. gambiense*, the cell protoplasm when well stained is homogeneous, and there appear constantly in the blood films a few very minute parasites measuring as little as 8μ in length. Some of the short and stumpy parasites have the nucleus situated far forwards at the anterior (flagellar) end of the body. The occurrence of a few peculiar trypanosomes which appear to have a flagellum free in its whole length is also remarkable.

Considering the morphological features of the parasite, and the peculiar symptoms of the disease produced by it, I am convinced that this trypanosome from Nigeria cannot be regarded as belonging to the same species as *T. gambiense*. I therefore propose for it the name *T. nigeriense*.

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ADDENDUM ET CORRIGENDUM

The following Plate (Pl. XXVIII) is of *Trypanosoma nigeriense*, Macfie, 1913, and illustrates the preceding paper, already published.

In the text of Dr. Macfie's paper the Plate was numbered inadvertently as XXXIII. Hence, on pp. 344-346 the Plate should have been referred to as Pl. XXVIII.

EXPLANATION OF PLATE XXVIII

Trypanosoma nigeriense (Macfie, 1913)

Figs. 1 to 23. Forms of the trypanosome seen in the blood of a guinea-pig. × 2,100.

Figs. 1-5. Long forms.

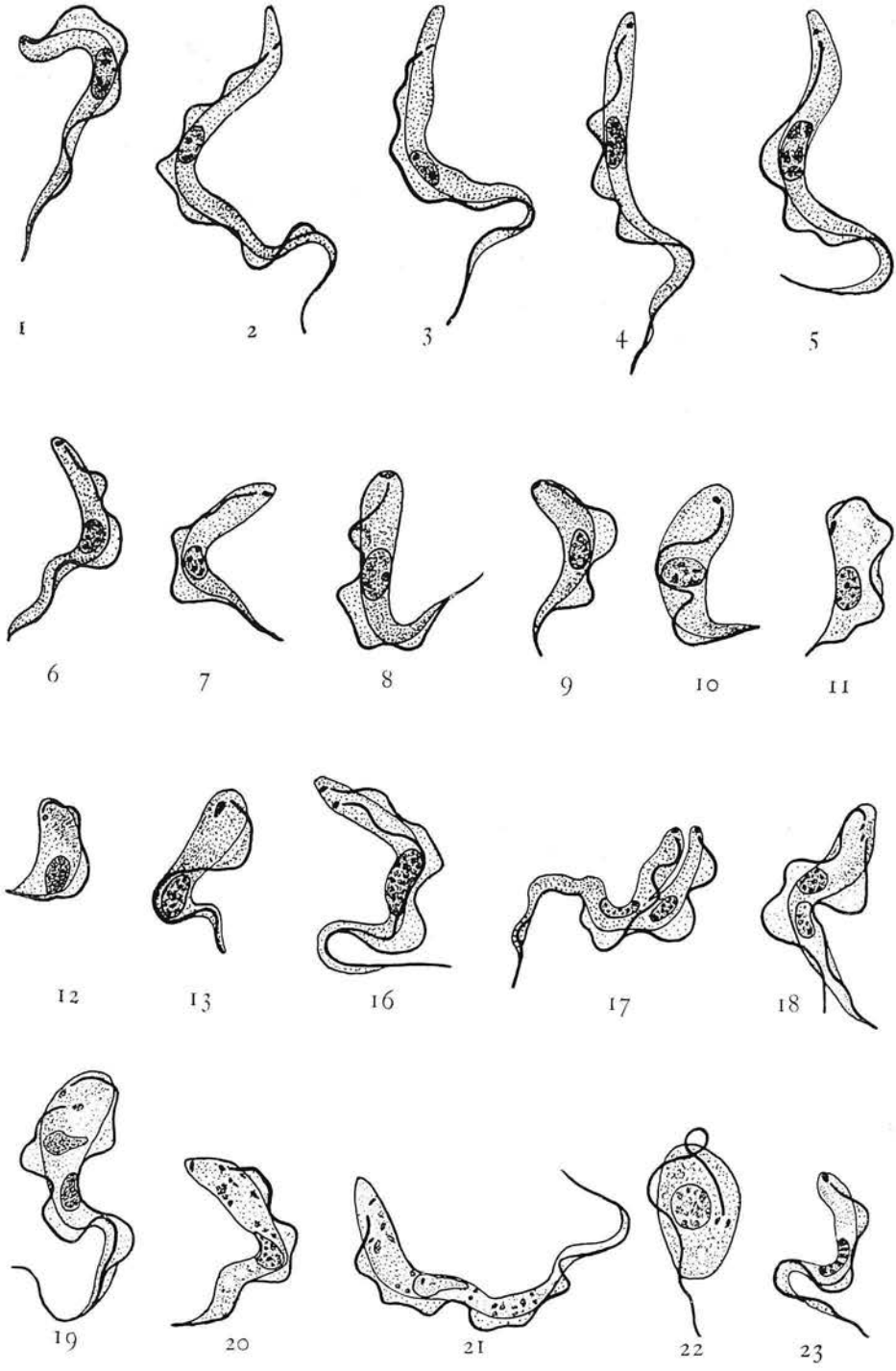
Figs. 6-9. Intermediate forms.

Figs. 10-13. Short, stumpy forms. In Fig. 13 the nucleus is seen to be near the anterior (flagellar) end.

Figs. 16-19. Dividing forms.

Figs. 20-21. Trypanosomes containing granules and some vacuoles.

Fig. 22. Rounded form.



TRYPANOSOMA NIGERIENSE

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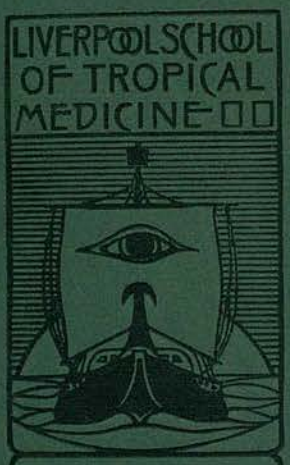

ANNALS

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On the Pathogenicity of the Trypanosome (*T. nigeriense*) from a Case of Sleeping Sickness from Eket, Southern Nigeria

J. W. SCOTT MACFIE

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ON THE PATHOGENICITY OF THE TRYPANOSOME (*T. NIGERIENSE*) FROM A CASE OF SLEEPING SICKNESS FROM EKET, SOUTHERN NIGERIA

BY

J. W. SCOTT MACFIE, M.A., M.B., Ch.B.

(WEST AFRICAN MEDICAL STAFF)

(*Received for publication* 20 *December*, 1913)

When describing, in a former paper, certain morphological features of a trypanosome obtained from a case of sleeping sickness at Eket in Southern Nigeria,[1] I called attention to the fact that the parasite appeared to have a low degree of virulence when inoculated into the smaller laboratory animals, and added a short table embodying my results up to the time of writing. Since then I have continued the investigation of the animal reactions of this trypanosome, and the following is an account of the results obtained.

Considerable difficulty was experienced in procuring a sufficient supply of animals for experimental purposes, and for this reason it was impossible to carry out as large a number of inoculations as I should have wished. Unfortunately no white mice were available, and until recently I was unable to obtain white rats. For experimental purposes wild rats and mice are not satisfactory owing to the difficulty of handling them; and moreover, the majority of the rats caught near Lagos are infected with *T. lewisi*. I regret also that it was necessary to discontinue these experiments somewhat prematurely, owing to the fact that I was leaving Lagos.

The strain of trypanosome used in the following experiments was obtained from a guinea-pig inoculated by the late Dr. Foran with cerebro-spinal fluid from one of the cases of sleeping sickness under his care at the isolation camp near Eket. All the first inoculations were made with the blood of this animal; but after its death, which occurred on the 158th day, blood from two other infected guinea-pigs, and one infected monkey, was used.

All the inoculations, with the exception of two, were subcutaneous, and the quantity of infective blood employed was in each case small. The latter precaution seemed necessary, in view of the experiences of Beck,[2] who explains the uncertain effects of inoculations with larger volumes of the infective blood to the presence of anti-bodies which act as trypanocides. The blood was diluted with 1 % sodium citrate solution before use.

In most of the animals which became infected trypanosomes were scanty. In no case did their number approximate to that found, for example, in a guinea-pig infected with *T. brucei* (*ugandae*). Parasites of the small type characteristic of the strain were seen, however, in all the animals in which the trypanosomes appeared in moderate numbers. None of the animals experimented with showed any symptoms, and in every case the disease was chronic. The appearance of the parasites in the peripheral blood of animals that were infected was inconstant; and on October 30th, the last day included for the purposes of this paper, all the examinations were negative.

RATS

Eight experiments were performed with rats; two with black rats, and six with white rats. Seven of the experiments were negative. In one animal a single trypanosome was found in the blood, on the 59th day. At a second examination on the same day, and on subsequent days, no parasites were found. Three of the rats died of intercurrent disease, but their tissues did not show any signs of trypanosomiasis. The other animals showed no sign of illness during the 48 to 69 days they were under observation.

TABLE I.—Rats inoculated with the *Nigerian strain.*

Animal	Inoculation	Day on which parasites were first seen in the blood	Day on which death occurred	Remarks
Black rat, No. 38	Subcutaneous from guinea-pig, No. 1	—	7th	Parasites never found in the blood. Died of intercurrent disease on the 7th day.
Black rat, No. 51	Subcutaneous from guinea-pig, No. 1	—	10th	Parasites never found in the blood. Died of intercurrent disease on the 10th day.
White rat, No. 75	Subcutaneous from guinea-pig, No. 23	59th	—	A single trypanosome seen once (on the 59th day). Animal alive and well after 69 days.
White rat, No. 76	,,	—	—	Parasites never found in the blood. Animal alive and well after 69 days.
White rat, No. 77	,,	—	—	,, ,,
White rat, No. 117	Subcutaneous from guinea-pig, No. 22	—	14th	Parasites never found in the blood. Died of intercurrent disease on the 14th day.
White rat, No. 118	,,	—	—	Parasites never found in the blood. Animal alive and well after 48 days.
White rat, No. 119	,,	—	—	,, ,,

MICE

Unfortunately, no tame mice were available, and it was, therefore, necessary to use the local wild mice (*Mus musculus*) for experimental purposes. These animals were difficult to handle, and were apt to be killed in catching them for examination. Seven experiments were carried out, three of which were positive, and four

negative. In those animals that were infected the parasites were always either scanty or absent. One animal, which was under observation 107 days, did not show trypanosomes after the 48th day. The incubation period was 5 to 9 days. The disease was chronic. The duration was at least 107 days, but as no animal died of the disease, it is not possible to state any definite period. The infected animals presented no symptoms of illness.

TABLE II.—Mice inoculated with the *Nigerian strain*.

Animal	Inoculation	Day on which parasites were first seen in the blood	Day on which death occurred	Remarks
Mouse, No. 7	Subcutaneous from guinea-pig, No. 1	—	30th	Parasites never found in blood. Died on the 30th day. Tissues showed no signs of trypanosomiasis.
Mouse, No. 24	Subcutaneous from guinea-pig, No. 1	5th	51st	Parasites very scanty or absent. Accidentally killed on the 51st day. Animal showed no symptoms of illness.
Mouse, No. 26	Subcutaneous from guinea-pig, No. 1	7th	11th	Parasites scanty. Accidentally killed on the 11th day.
Mouse, No. 32	Subcutaneous from guinea-pig, No. 1	—	—	Parasites never found in the blood. Animal alive and well after 121 days.
Mouse, No. 35	Subcutaneous from guinea-pig, No. 1	—	—	" "
Mouse, No. 44	Subcutaneous from guinea-pig, No. 1	9th	—	Parasites rarely found, and then only in very small numbers. None seen for nearly two months. Animal alive and well after 107 days.
Mouse, No. 45	Subcutaneous from guinea-pig, No. 1	—	—	Parasites never found in the blood. Animal alive and well after 107 days.

GUINEA-PIGS

Twelve experiments were carried out with guinea-pigs. Three of the animals became infected, nine did not. The disease was very chronic, and the animals presented no symptoms of illness. The parasites were fairly constantly present in the blood, but

usually in small numbers. They were never very numerous. The incubation period was 27 days. The duration was 158 days in the one animal that died of the disease.

TABLE III.—Guinea-pigs inoculated with the *Nigerian strain*.

Animal	Inoculation	Day on which parasites were first seen in the blood	Day on which death occurred	Remarks
Guinea-pig, No. 1	Subcutaneous from a case of sleeping sickness at Eket	(?)	158th	Parasites never present in large numbers, but generally fairly numerous.
Guinea-pig, No. 2	Subcutaneous from guinea-pig, No. 1	—	—	Parasites never found in the blood. Animal alive and well after 171 days.
Guinea-pig, No. 16	Intraperitoneal from guinea-pig, No. 1	—	16th	Parasites never found in the blood. Died of intercurrent disease. No evidences of trypanosomiasis in the tissues.
Guinea-pig, No. 17	Intraperitoneal from guinea-pig, No. 1	—	—	Parasites never found in the blood. Animal alive and well after 149 days.
Guinea-pig, No. 22	Subcutaneous from guinea-pig, No. 1	27th	—	Parasites often scanty or absent. Sent home to England on the 124th day. Animal at that time alive and well.
Guinea-pig, No. 23	Subcutaneous from guinea-pig, No. 1	27th	—	Parasites never very numerous and often either scanty or absent. Animal alive and apparently well after 139 days.
Guinea-pig, No. 120	Subcutaneous from guinea-pig, No. 22	—	—	Parasites never found in the blood. Animal alive and well after 48 days.
Guinea-pig, No. 121	Subcutaneous from guinea-pig, No. 22	—	—	Parasites never found in the blood. Animal alive and well after 48 days.
Guinea-pig, No. 122	,,	—	—	,, ,,
Guinea-pig, No. 123	Subcutaneous from monkey, No. 21	—	39th	Parasites never found in the blood. Died on the 39th day of intercurrent disease. No signs of trypanosomiasis in the tissues.
Guinea-pig, No. 124	,,	—	—	Parasites never found in the blood, Animal alive and well after 48 days.
Guinea-pig, No. 125	,,	—	—	,, ,,

DOGS

Seven experiments were carried out with dogs. All proved refractory except one. This animal first showed trypanosomes on the 85th day. On subsequent days parasites were generally to be found in the blood in small numbers, sometimes, however, they appeared to be absent. The dog showed no symptoms of disease, and was still alive and in normal health on the 115th day.

TABLE IV.—Dogs inoculated with the *Nigerian strain*.

Animal	Inoculation	Day on which parasites were first seen in the blood	Day on which death occurred	Remarks
Dog, No. 13	Subcutaneous from guinea-pig, No. 1	—	93rd	Parasites never found in the blood. Animal died on the 93rd day of distemper and mange.
Dog, No. 14	Subcutaneous from guinea-pig, No. 1	—	—	Parasites never found in the blood. Animal alive and well after 149 days.
Dog, No. 36	Subcutaneous from guinea-pig, No. 1	85th	—	Parasites never numerous. Animal alive and apparently in good health after 115 days.
Dog, No. 106	Subcutaneous from guinea-pig, No. 23	—	—	Parasites never found in the blood. Animal alive and well after 55 days.
Dog, No. 107	,,	—	—	,, ,,
Dog, No. 108	,,	—	—	,, ,,
Dog, No. 109	,,	—	—	,, ,,

MONKEYS

Only two experiments were performed owing to the difficulty of obtaining monkeys. The two animals were different, the one being a small red-haired monkey, and the other, also red-haired, a somewhat larger species. Both were inoculated with blood from the original guinea-pig (No. 1) obtained from Eket. The disease ran a chronic course, and at the time of writing both animals are alive, and appear to be in normal health. Trypanosomes first appeared in the peripheral blood on the 8th and 11th days after inoculation. No symptoms of disease have as yet manifested themselves, although it is now 118 and 137 days respectively since the injections were made. Throughout the course of the disease, so far as it has at present advanced, the trypanosomes have been scanty, and it has often been impossible to find them at all. In one

(No. 37) no trypanosomes were detected for over a month, namely, from the 53rd to the 90th day.

TABLE V.—Monkeys inoculated with the *Nigerian strain.*

Animal	Inoculation	Day on which parasites were first seen in the blood	Day on which death occurred	Remarks
Red-haired monkey, No. 21	Subcutaneous from guinea-pig, No. 1	11th	—	Parasites always scanty or absent. Animal alive and well after 137 days.
Small red-haired monkey, No. 37	,,	8th	—	Parasites always scanty or absent. Animal alive and well after 118 days.

GOATS

Two experiments were carried out with goats. One became infected. The disease was very chronic. Trypanosomes were only found in the blood on two occasions, namely, on the 63rd and 65th days, and were present in very small numbers. The goat was still alive on the 149th day, and appeared to be in normal health. No symptoms of disease were observed.

TABLE VI.—Goats inoculated with the *Nigerian strain.*

Animal	Inoculation	Day on which parasites were first seen in the blood	Day on which death occurred	Remarks
Goat, No. 11	Subcutaneous from guinea-pig, No. 1	63rd	—	Parasites only found twice, on the 63rd and 65th days, in very small numbers. Animal alive and well after 149 days.
Goat, No. 12	Subcutaneous from guinea-pig, No. 1	—	94th	Parasites never found in the blood. Animal died on the 94th day of a disease of the liver.

The above experiments indicate the slight degree of pathogenicity of the Nigerian trypanosome. Monkeys, guinea-pigs, and mice appeared to be the most susceptible animals, but even in them the disease was of an exceptionally chronic type.

Dogs were infected only with difficulty, and rats and goats seemed to be even more resistant. Of the 38 animals inoculated so far, only one has died of trypanosomiasis, and only ten have been infected. In some of the latter trypanosomes have not been found in the blood during the last two months.

TABLE VII.—Results obtained by various workers

	Yorke		Royal Society Commissioners in Uganda		Thomas and Linton	
	Incubation	Duration	Incubation	Duration	Incubation	Duration
	Days	Days	Days	Months	Days	Days
Rat	4	14	20	3–8+	8	45–388
Mouse	—	—	—	—	4–7	11–14
Guinea-pig	15	3–5+ months	—	—	12–45	3–8 months
Rabbit	12	3–5+ months	—	—	5–15	54–128 days
Dog	14	More than 1½ months	22	Some months	5–10	33–43 days
Monkey—						
Macacus rhesus	4	1–3	9–22	2–8	—	—
Cercopithecus callitrichus	13	3–5 months	9–40	2½–12 months	—	—
Cercopithecus ruber ...	—	—	—	—	—	—
Goat	Parasites only found once on 19th day in Goat 1; never found in Goat 2	3–5+ months	Parasites found on 13th day after 5th inoculation in one goat. Other two goats negative	One animal died on 22nd day. Other two recovered	—	All died; duration not stated
Donkey	—	—	Three were inoculated, but did not become infected	—	Parasites found on rare occasions	—
Horse	—	—	—	—	30 days	Recovered

G. – *T. gambiense*, from Gambia.
U. – Tryp. of Sleeping Sickness in Uganda.
K. – Tryp. of Sleeping Sickness in Congo.

with different strains of human Trypanosomes.

Laveran		Brumpt and Wurtz		Beck		Bentmann and Günther	
Incubation	Duration	Incubation	Duration	Incubation	Duration	Incubation	Duration
Days G. 13 U. 11 K. —	Days 62 77 81	Days 3–4	Months 4–5	Days 3–5	Months 3–4	Days U. 4·9 K. 12·5	Days 69 81
G. 13 U. 8–20 K. 30–45	57 153–216 281–351	2–3	3–4	2–3	8–10 days	U. 5·3 K. 4·9	49 90 —
G. 26 U. 16 K. 20	100 81 100	Always negative	1½	2–3	3–5 months	U. 6 experiments with no positive result. K. Only 2 positive results out of 21 experiments.	
—	—	Always negative	1–4+	8–14	4–5 months	U. 9 days K. 9	28·6 days 49
—	—	17	66 days	2–3	1½–4 months	—	—
—	—	8–9	16 days to 2 months	—	—	—	—
—	—	7	19-88+ days	—	—	U. 10 K. 12	82 105
—	—	7	24	—	—	—	—

Very dissimilar results have been obtained by different authors working with various strains of *T. gambiense*. I have been unable, in Nigeria, to consult all the original papers on this subject; but the summary given by Yorke,[3] and reprinted above (Table 7), shows clearly how greatly the virulence may vary. The results of my experiments with the Nigerian strain are also shown in tabular form (Table 8) for comparison with those of other investigators.

TABLE VIII.—Results of inoculations with the *Nigerian strain*.

Animal	Incubation	Duration	Remarks
Rat	59 days	69 + days	A single trypanosome seen once, on the 59th day, in one out of eight animals inoculated.
Mouse ...	5–9 days	107 + days	Three positive results out of seven experiments.
Guinea-pig	27 days	158 days	Three positive results out of twelve experiments.
Dog ...	85 days	115 + days	One positive result out of seven experiments.
Monkey ...	8–11 days	137 + days	
Goat ...	63 days	149 + days	

Several conditions affect the results of inoculations into animals. The virulence may be modified by a number of passages through the same species (Laveran,[4] Uhlenhuth,[5] &c.), inoculations may fail owing to the insufficient dilution of the infective blood (Uhlenhuth, Huebener and Woithe) or to too large a quantity being employed, and individual animals of the same species vary greatly in their susceptibility. If, as must be assumed, all the strains employed were of the same species, the different results obtained can only be explained by supposing that in some cases modification of the virulence had occurred in some such manner.

The strain of *T. gambiense* used by Yorke[6] was much more pathogenic than the Nigerian trypanosome to practically all the animals with which experiments were performed. His strain had been obtained from a case of human trypanosomiasis from the French Congo, in 1905, and had been maintained by passages through rats. It was said to have 'gradually become more virulent for rats.' The Nigerian trypanosome differs from all the other strains in its slight degree of pathogenicity to rats and dogs. Its virulence is unlike that of any of the strains of *T. gambiense* mentioned in the above summary, a fact which furnishes additional evidence in favour of considering it a new species of human trypanosome.

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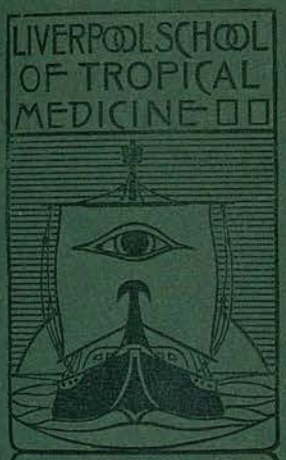
With the Authors Compliments



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Preliminary Note on the Development of a Human Trypanosome in the Gut of *Stomoxys nigra*

J. W. SCOTT MACFIE, M.A., M.B., Ch.B.

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PRELIMINARY NOTE ON THE DEVELOPMENT OF A HUMAN TRYPANOSOME IN THE GUT OF *STOMOXYS NIGRA*

BY

J. W. SCOTT MACFIE, M.A., M.B., CH.B.

WEST AFRICAN MEDICAL STAFF

(*Received for publication* 14 *September*, 1913)

The rôle of *Stomoxys* in the transmission of trypanosomiasis has been much discussed. The Sleeping Sickness Commission of the Royal Society performed many experiments to determine whether this insect could transmit *T. pecorum*. All the experiments were unsuccessful, and Sir David Bruce and his collaborators concluded that 'It will therefore require very convincing proof to bring this Commission to the belief that *Stomoxys* are carriers of this disease.'[1] A similar conclusion was arrived at by Bevan,[2] in Southern Rhodesia, who failed to infect sheep with the Hartley trypanosome by means of *Stomoxys* flies. On the other hand, Bouet and Roubaud,[3] in French West Africa, succeeded in transmitting *T. cazalboui*, *T. pecaudi*, *T. sudanense*, and *T. evansi* by means of *Stomoxys* (probably *S. calcitrans*). They did not, however, observe any developmental forms in the digestive tract or proboscis of the insect, and although they considered the species of fundamental importance in the etiology of trypanosomiasis, they were not able to regard it as a typical intermediary host. In Northern Nigeria also this genus has fallen under suspicion, and the writer has recorded one case in which an infection with *T. brucei* (*pecaudi*) was probably conveyed to a horse by *Stomoxys nigra* or *Stomoxys calcitrans*.[4]

Last May, the writer had an opportunity of experimenting with flies of this genus at the Medical Research Institute, Lagos. As he succeeded in breeding *Stomoxys nigra* in captivity, it was hoped to investigate thoroughly the rôle of this insect in the transmission of trypanosomiasis. The experiments were however interrupted, and

as it seems unlikely that they can be resumed for some time, the following incomplete records are given by way of a preliminary note.

Towards the end of May, and at the beginning of June, 1913, a number of *Stomoxys nigra* flies, caught in the laboratory, were fed on a guinea-pig infected with the trypanosome from a case of sleeping sickness from Eket in Southern Nigeria. The morphology of this trypanosome, which is being described elsewhere, differed in several respects from that of *T. gambiense*. Thirteen of the flies were dissected from one to six days after the first infecting feed. In six of them flagellates (Herpetomonas) were found in the mid-gut. As a control, twelve flies that had not fed on the infected animal were dissected. No flagellates were found in them.

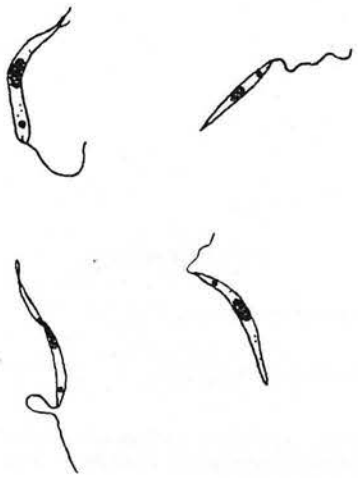
Unfed Flies			Fed Flies		
Number Dissected	With flagellates in the gut	Without flagellates in the gut	Number Dissected	With flagellates in the gut	Without flagellates in the gut
12	0	12	13	6	7

From these observations it was thought probable that the presence of the flagellates in the mid-gut must be due to the infecting feeds on the guinea-pig. As, however, it was possible that the flies might have been naturally infected, experiments were begun with flies hatched out in the laboratory.

Experiment 1. On June 14th a *Stomoxys nigra* fly that had hatched out on the previous day was fed on the infected guinea-pig. On June 17th the fly was killed and dissected. Herpetomonas were found in the mid-gut. No flagellates were present in the salivary glands and proboscis. The attached sketches, drawn with a camera lucida, illustrate some of the flagellates found in the mid-gut of this fly.

Experiment 2. On June 28th a *Stomoxys nigra* fly which had hatched out the previous day was fed on the infected guinea-pig. On July 2nd the fly died, and was dissected. Herpetomonas were found in its mid-gut. No flagellates were seen in the salivary glands and proboscis.

Each fly received only a single infecting feed. Two other specimens of *Stomoxys nigra* that had been bred out in the laboratory, but had not been fed on the infected guinea-pig, were dissected. No flagellates were found in either of them.



Herpetomonas (× 1000) from gut of *Stomoxys nigra*.

At this stage the experiments had to be abandoned. Some apology is necessary for recording such incomplete observations. The two experiments given above were, however, quite definite, and would seem to prove that the trypanosome with which the guinea-pig was infected was capable of development in the gut of *Stomoxys nigra*. I am not aware that this fact has previously been established, and its importance must be my excuse for publishing this note. In Nigeria, in the native towns and European stations, *Stomoxys* flies abound. If, as appears probable, they are capable of serving as the intermediary host of human trypanosomiasis, they deserve greater consideration than they at present obtain.

MEDICAL RESEARCH INSTITUTE,
LAGOS,
SOUTHERN NIGERIA,
August, 1913.

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J. W. SCOTT MACFIE, M.A., M.B.
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G. H. GALLAGHER, L.R.C.S., L.R.C.P.

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SLEEPING SICKNESS IN THE EKET DISTRICT OF NIGERIA

BY

J. W. SCOTT MACFIE, M.A., M.B.

AND

G. H. GALLAGHER, L.R.C.S., L.R.C.P.

WEST AFRICAN MEDICAL STAFF, NIGERIA.

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PLATES XIX—XXIII, AND MAP

Although the Eket district is by no means one of the least accessible regions of Southern Nigeria, and has been the field for the activities of the Qua Iboe mission for a quarter of a century, it was not until recently that the presence of sleeping sickness was suspected. This fact is no doubt accounted for by the peculiarly mild form assumed by the disease, which at first sight would not suggest the malady which has devastated the Congo and Uganda. Indeed, but for the acuteness of the observation of Mr. W. C. W. Eakin, of the Qua Iboe mission, it might have remained unidentified for many years longer. Eight years ago Mr. Eakin's suspicions were first aroused by the appearance of a sick woman at Ikotobo, and in 1911, another case having come under his notice, he decided that the matter should be reported officially. Mr. S. A. Bill accordingly communicated with the medical officer at Calabar, and early in 1912 Dr. R. W. Gray visited the district for the purpose of investigating the subject. In May Dr. Gray was able to report that he had found trypanosomes in the gland juice of several patients, thus confirming Mr. Eakin's suspicions; and as the disease was found to be prevalent throughout the neighbourhood, it was decided to establish an isolation camp at Ikotobo,* where the patients might undergo treatment.

*The name of this town, which is generally pronounced Ikorobo, is more correctly spelt Ikotobo.

At our suggestion Mr. Eakin has very kindly written an account of his observations, which is included as an appendix to this paper (Appendix I). He has also recorded the native views on sleeping sickness in a manner that could only have been done by one who spoke their language, and had won their esteem and trust by long years of devoted service. His account is of considerable interest, and will serve to illustrate how deeply a consciousness of the presence of sleeping sickness has influenced the lives of the people. Some idea of the importance attached to the disease by the natives may be gathered from the fact that their chiefs not only voluntarily gave the land for the camp, but also erected all the buildings, including a house for a medical officer, a hospital and dispensary, and accommodation for about 130 patients.

The task of investigating the disease and starting the isolation camp was entrusted to the late Dr. P. F. Foran. In carrying out this work he displayed untiring energy, and during the year he was in Eket succeeded in winning the confidence of the people. His work can best be judged by its results, and the ready way in which the natives have availed themselves of the advantages afforded them at Ikotobo is eloquent testimony to his success. But for his untimely death, which occurred on the voyage back to England, he would no doubt have written an account of sleeping sickness as he found it in the Eket district. This unfortunately is now impossible. We believe, however, that a description of the disease, which presents certain unusual features, will be of interest; and although we cannot hope to give as complete an account as could have been given by Dr. Foran, we take this opportunity of expressing our indebtedness to his careful investigation, and our deep regret that he was unable himself to record the results of his observations.

PHYSICAL AND OTHER FEATURES OF THE EKET DISTRICT

The Eket district, a division of the Eastern province of Southern Nigeria, occupies the tongue of land lying between Calabar and Opobo. It is situated just north of 4° 30′ North latitude, and is divided into two unequal portions by the 8th degree of East longitude. To the north it is bounded by the districts of Calabar and Uyo, to the west by Opobo, to the south by the sea,

and to the east by the Cross River. The area of the district is estimated as 690 square miles (see Map).

The country is well watered by creeks and streams throughout its extent, and in the wet season the southern portions, which are low lying and at other seasons swampy, are under water. This part of the district is forest clad, and except for a few fishing villages on the coast, is practically uninhabited. The Qua Iboe River traverses the western portion of the district on its way to empty itself into the sea, and is joined by the Obium River (Pl. XIX, fig. 2) just above the town of Eket, the headquarters of the district. The Obium River drains the central and south-western portions of Eket, and is a stream of considerable size. The towns and villages on the banks of this river, and of its tributary streams, are those in which sleeping sickness appears to be most prevalent.

The vegetation all over the country is either thick scrub, or forest in which palm trees are abundant. The villages consist of a larger or smaller number of compounds separated, and often hidden from one another by dense bush; so that although they are in fact innumerable, it is possible to pass for considerable distances along the main roads without seeing a single one (Pl. XIX, fig. 1). There are no exceptionally large towns, but rather a great number of small towns and villages. The total population of the district, according to the census of 1911, is estimated at about 184,000—that is the average density of the native population per square mile is 266·64. The chief tribes are in order the Ibibio, the Eket, and the Oron.

The climate, as would be expected, is humid. Meteorological observations have not been recorded, but it may be assumed that they would have been similar to those of Calabar, where the average rainfall is 147·3 inches, the average maximum temperature 88·3° F., the average minimum 71° F., and the mean monthly humidity 88·1.

Sheep, goats, and dogs are common throughout the district, and in most of the towns and villages a few dwarf cattle are to be found. These domestic animals appear to thrive. Larger cattle and horses are unknown. In some places pigs are kept also, but as they are considered unclean animals, and are forbidden by

native law, their presence is often concealed. Game is rare in most parts of the district, as might be expected, considering the density of the population. Small antelope are occasionally seen, however, and in the southern areas elephants even may be met with. Monkeys are common in some places, especially in the forests along the river banks.

Biting flies are fairly numerous. Tabanidae are abundant on the Qua Iboe River, and *Chrysops dimidiata* has been taken in several localities. Tsetse flies are widely distributed over the district, but seldom occur in large numbers. During a visit of two months to Ikotobo one of us (J. W. S. M.) never saw a single specimen at liberty, although the house he occupied was situated close to a small stream with thickly-wooded banks that appeared to be an ideal haunt for *Glossina palpalis*, and was within a yard or two of the main road through the district along which a constant procession of natives passed all day. A specially trained collector was employed to catch tsetse flies, but he seldom succeeded in bringing back more than half a dozen as the result of a day's search, and in order to obtain these he had often to go long distances into the bush.

During the months of November and December practically all the tsetse flies caught near Ikotobo were *G. tachinoides*. Only two specimens of *G. palpalis* were taken, and a single example of *G. caliginea*, a male, was obtained from near Eket. *G. tachinoides* is unquestionably the most common species throughout the neighbourhood of Ikotobo, which is at the centre of the sleeping sickness area. The natives are familiar with tsetse flies, and affirm that they are most troublesome in the bush, and not, as might be expected considering the nature of the country, at the waterside. This observation corresponds with the fact that *G. tachinoides* appears to be the predominant species, and that *G. palpalis* is relatively rare. A point of some interest is that *G. tachinoides* is particularly easy to obtain where pigs are found, and is often seen attacking these animals. The natives themselves regard pigs as unclean, and bearing in mind their recognition of the fact that biting flies follow these animals (see Appendix 1), it seems possible that the reason for this belief may have something to do with the association with tsetse flies. The people are not Mohammedans.

In this connexion it is of interest to note that Moiser (1913) in writing of the haunts and habits of *G. tachinoides* in Bornu, Northern Nigeria, states .' Another noticeable feature of all the belts I have examined is the large number of warthogs to be found in and around them.'

G. tachinoides is exceedingly common in Northern Nigeria, but has hitherto been recorded from very few localities in Southern Nigeria. Simpson (1912) mentions only two, Ikom and Bende, but remarks that its occurrence at the latter station 'would seem to indicate that it may be found more commonly in the Cross River basin than is supposed.' In dealing with the distribution of the various species of *Glossina*, the same writer says 'The conditions which are most favourable for *G. palpalis* are, generally speaking, most unfavourable for *G. tachinoides*. Where the country is open, the vegetation sparse, the dry season well defined and the rainfall slight, there *G. tachinoides* is most abundant.' It is somewhat remarkable therefore that *G. tachinoides* should be so common in Eket, where the dense vegetation, the humidity of the atmosphere, and the heavy rainfall would appear to be more suitable for *G. palpalis*. Roubaud (1911), however, while recognising that in Upper Dahomey *G. tachinoides* represents the *G. palpalis* of the more southerly regions, lays emphasis on the fact that both species are hygrophilous, and need the constant humidity of the forest belts, and that it is its resistance to heat alone which enables *G. tachinoides* to overlap northwards the area of *G. palpalis*.

AETIOLOGY OF THE DISEASE

Sleeping sickness has undoubtedly existed in the Eket district for a great number of years. The natives are unanimous in stating that it has been familiar to them all their lives; but some of them assert that during recent years its incidence has increased owing to the neglect by the present generation of the traditional laws and customs relating to the behaviour of infected persons. Whether this is in fact the case cannot be determined with certainty, but that these laws exist is in itself a proof of the antiquity of the disease. Tradition moreover affirms that there was a time, long ago, when the disease was a much more serious

scourge than it is at present, when whole villages were exterminated by it.

In the first appendix to this paper a full account is given by Mr. Eakin of the history and traditions derived from native sources. It will be unnecessary to repeat any of this matter since the subject is there dealt with in such an admirable manner.

During the sixteen months in which sleeping sickness has been under investigation, 222 cases have been identified in which the trypanosomes have been demonstrated. In addition 114 cases have been met with presenting some of the clinical features of the disease but in which the parasites have not actually been found. There can be little doubt that the majority of these were actually cases of trypanosomiasis. In what follows no account has, however, been taken of them, only those cases being considered in which the clinical diagnosis has been confirmed by the aid of the microscope. Eighty-four of the cases have been studied by us personally, but for the details of the remainder we have relied on the notes in the case-books at the camp, which for the most part were recorded by Dr. Foran.

Cases have been detected in every part of the district, but a glance at the accompanying map, on which the towns in which sleeping sickness has occurred are specially marked, will show that the disease is most prevalent in the neighbourhood of Ikotobo, and in the areas drained by the Obium River. Beyond the bounds of Eket the occurrence of sleeping sickness has not been investigated. There can be little doubt that the disease will be found to extend to some extent at any rate into the neighbouring districts, but as this fact has not yet been established, and as we are not in a position to state that the disease occurs in an identical form elsewhere, for the purposes of this paper we shall limit our observations strictly to those cases studied at Ikotobo, all of which had come from the Eket district.

THE INFLUENCE OF SEX AND AGE

Sex

Todd and Wolbach (1911) found that in the Gambia the numbers of men and women suffering from sleeping sickness were approximately equal, but that more boys than girls were infected. In the Congo, in 1903-05, the figures were somewhat different. In

a recent paper Todd (1913, see Table I) has tabulated the cases examined according to sex and age, and it appears that many more men and boys (63·2 % and 6·7 %) were found to be infected than women and girls (26·8 % and 2·6 %). Sanderson (1912) and Hearsey (1912) in Nyasaland have also recorded a much higher percentage of cases in males than in females, the latter stating that 'the number of males diagnosed as infected is nearly twice that of females.'

In Eket also males appear to be more affected by the disease than females. Of the 222 cases in which the diagnosis has been confirmed by the discovery of trypanosomes, 140 (or 63 %) were males, and 82 (or 37 %) females. As in the Gambia the numbers of men and women were approximately equal (24·3 % and 19·4 % respectively), but many more boys (38·7 %) than girls (17·6 %) were infected.

To what extent these figures represent the actual distribution between the sexes it is difficult to decide. The natives themselves assert that the disease is as common in females as in males. Todd (1913) records that in the Gambia 'glandular enlargement is common among children of both sexes, but especially in boys—probably because boys are less careful of their person than are girls.' As will be pointed out later, the majority of the patients in Eket are children, and it has been mentioned that glandular enlargement is, in the eyes of the natives, almost pathognomonic of the disease. It is possible that boys, if they are really less careful of their persons, may be more liable than girls to the impetigo of the scalp that is so common in Eket, and that this may cause a greater number of them to exhibit enlargement of the cervical glands. This in turn would account for a larger number of boys being brought under observation than girls.

If this is a true explanation it is possible that an even greater number of children may be infected than the statistics show; and that only those cases are brought for treatment, and are thus identified, which from one cause or another have a considerable degree of glandular enlargement.

Another possible explanation suggests itself. Impetigo of the scalp is exceedingly common amongst the children, and in not a few cases, in which a clear history has been elicited, has been stated

to have preceded the symptoms of sleeping sickness. It is possible therefore that the affection of the glands following the appearance of the impetigo may enable the trypanosomes to establish themselves in the subject, and that the boys, being less scrupulously clean, may be more liable to this inroad of infection. On the other hand the condition of the scalp and skin may have been secondary to the slight abrasions to which children are subject. For it has been observed by Borchers (1912) that the tissues of patients suffering from sleeping sickness are unable to protect themselves against infection.

AGE

In Tables 1 and 2 the 222 cases of sleeping sickness are distributed according to sex and age in the manner suggested by Todd (1913). In explaining his reasons for adopting these unusual age periods, which are different for the two sexes and do not contain an equal number of years, this author states that they 'were chosen because they represent, approximately, the periods during which male and female natives follow the occupations of children, adults and elderly people.' 'Therefore,' he adds, 'in comparing the figures . . . . . it must be remembered that errors in compilation have occurred, and that the span of years, allotted by the classification employed, is for adults almost thrice that apportioned to children; consequently, a meaning can be attached only to very considerable and constant differences in the figures stated for each class.' We have adopted the same age periods both because we wished to compare our figures with those given by Todd for the Congo and the Gambia; and because the classification, based on occupation, is in our opinion a convenient one. It is often difficult to estimate the age with accuracy, and little assistance is to be obtained from the natives themselves who have but an imperfect conception of the longer intervals of time. Custom, however, arrogates certain offices to the children and the aged, and by bearing these in mind, together with the physical appearances, it is possible to make a fairly accurate guess at the age of every individual. In the graph (Chart 1) showing the distribution of the cases in periods of five years a more exact determination of the ages of the patients is, however, attempted.

TABLE 1.—Analysis of the cases of trypanosomiasis seen in Eket, and a comparison with the figures for the Congo and the Gambia.

SEX	MALE			FEMALE			TOTALS	
Age	0—13	14—44	45—	0—11	12—39	40—	Per-centages	Number of cases seen
Congo, 1903-05, selected populations	6·7%	63·2%	0·7%	2·6%	26·8%	0·0%	100%	269
Congo, 1903-05, unselected populations	6·1	57·2	2·0	2·0	32·7	0·0	100	147
Gambia, 1911	16·4	39·3	0·0	7·6	36·7	0·0	100	79
Eket district of Nigeria ...	38·7	23·9	0·4	17·6	19·4	0·0	100	222

TABLE 2.—Analysis of the cases of trypanosomiasis seen in Eket, and a comparison with the figures for the Congo and the Gambia.

COUNTRY	AGE			SEX	
	Children	Adults	Aged	Male	Female
Congo, 1903-05, combined populations	8·7%	89·95%	0·35%	67·95%	32·05%
Gambia 1911	24·0	76·0	0·0	55·7	44·3
Eket district of Nigeria	56·3	43·3	0·4	63·0	37·0

On comparing the figures for Eket with those for the Congo and the Gambia there is seen to be one outstanding feature, namely, the very large percentage of the cases that occurred in children. Over 56 % of the cases of trypanosomiasis seen in the Eket district were in children. The corresponding figure for the Gambia is

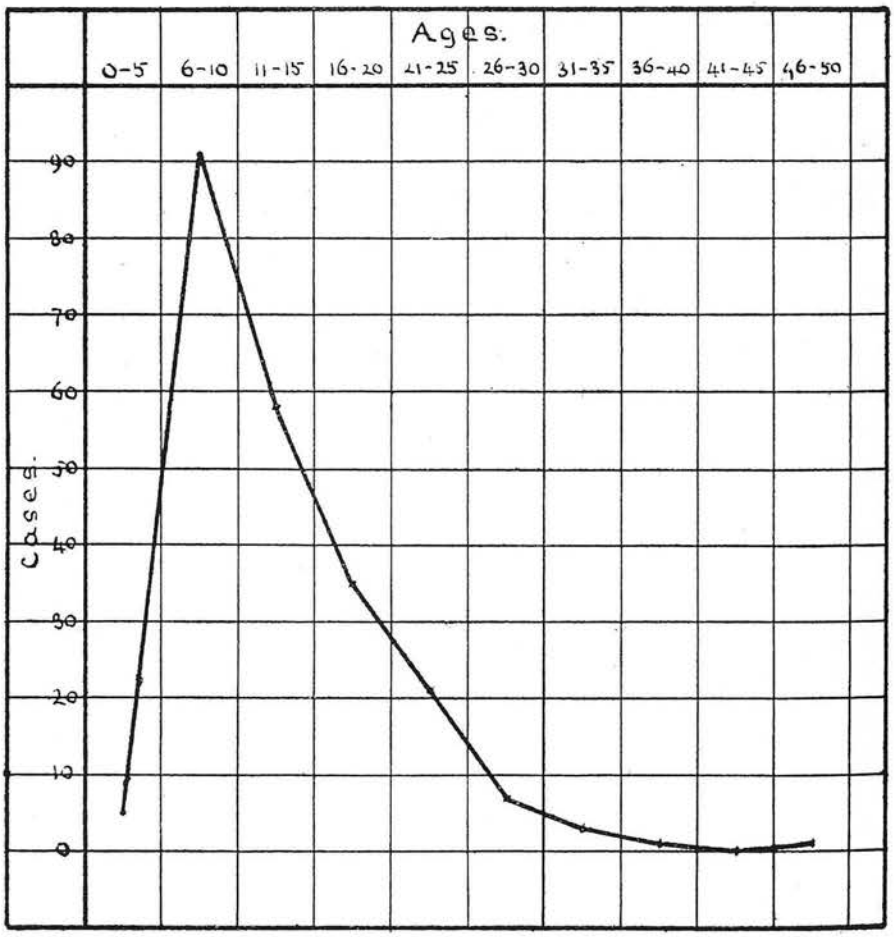


CHART I—Graph showing the ages, grouped in periods of five years, of 222 cases of trypanosomiasis identified in the Eket district of Southern Nigeria in 1912-13.

given by Todd as 24 %; and for the Congo 9·3 % or 8·1 %, according as the population from which the cases were drawn was a selected or an unselected one. Such a degree of disparity can hardly be explained by errors of compilation. In Eket, as has

already been explained, the natives are only too willing to avail themselves of the treatment afforded them at the isolation camp, and seldom offer any objection to examination. Indeed, they are ready to submit even to the excision of a gland for diagnostic purposes, as this form of treatment is that adopted by their own 'medicine men.'

Since natives of all ages and both sexes are accustomed to frequent the camp, there is no reason why the percentage of cases in children should be so high unless the disease is really most prevalent amongst them. It might even have been expected that relatively few children would have been brought for treatment, either from a natural distrust of strangers and foreigners, or because their number was actually small owing to the impotence that is an early symptom of the disease in adults.

Why then should the children be especially affected? One possible explanation may be found in the fact that they are the water carriers and the collectors of firewood, and being mostly unclad, are thus exposed to the attacks of the tsetse flies at the waterside and in the bush (Pl. XXI, fig. 8). Even the infants are not completely protected from the chance of infection since, slung on their mothers' backs, they are carried out into the cultivated lands where *G. tachinoides* is frequently found. Several of the infected adults were collectors of palm-wine, and therefore spent much of their time in the forests or thick bush. But whether they be farmers, traders, or what-not, and irrespective of sex and age, it may be assumed that all are exposed to the attacks of tsetse flies, especially to those of *G. tachinoides*, the species which is not only the most common, but also the frequenter of the bush and the partly cultivated grounds.

Although the majority of the cases were children, the range of age of the patients was considerable. At the time of writing (December 31st, 1913), the youngest inmate of the camp was $2\frac{1}{2}$ years of age, and the oldest 50. A closer analysis shows that 67·1 % of the cases occurred between the ages of 6 and 15 years, and that 85·1 % were in persons under 21.

DIAGNOSIS

Diagnosis has presented unusual difficulties. The majority of the cases brought to the camp have shown very slight symptoms, and would have been unrecognisable clinically. As a rule there was some degree of glandular enlargement, but as many of the children in the native towns have dirty heads, and as in a tropical country there are many causes which may produce this condition, it was impossible to base a diagnosis on this single sign. It is probable that the parents of the patients often observed other symptoms that would not be apparent to strangers, such as the change of character mentioned in the Report of the French Commission (1909). Their diagnosis indeed was seldom at fault. For this reason, and because it was inadvisable that the natives should be discouraged in any way from bringing in cases, a confirmation of the diagnosis by the finding of the parasites was a matter of importance. In practice, however, the trypanosomes were extremely difficult to detect, and in all the cases that came under our notice were rare. Dr. Foran, earlier in the year, noted that in several instances trypanosomes had been numerous in gland juice, a fact that may perhaps be accounted for by the most pronounced cases having been brought under his notice, since he was the first to make a systematic search for them.

The methods of diagnosis employed were the examination of thick and thin blood films, and of gland juice obtained either by puncture or excision of one of the enlarged cervical glands. Centrifugalization of blood and cerebro-spinal fluid could not be employed owing to lack of the necessary apparatus. Inoculation into susceptible animals was only carried out in one or two cases, as experimental animals were difficult to obtain, and because, as has been shown elsewhere, the trypanosome causing the sleeping sickness in Eket has a low degree of virulence, and often fails to infect the most convenient and procurable animals for the purpose, namely guinea-pigs. Monkeys, which might have proved of considerable value, were unobtainable.

By blood examination

Very variable results have been obtained from the examination of the blood in sleeping sickness by workers in different countries.

The members of the French Commission (1909), Martin, Leboeuf and Roubaud, found trypanosomes in 36-45 % of their cases in the French Congo and actually preferred blood examination to gland puncture for rapid diagnosis. Dutton and Todd (1906) in the Belgian Congo found parasites in the blood in 13·6 % of the cases. In the Gambia, however, Todd and Wolbach (1911) found trypanosomes in the blood in only eight out of 362 cases examined by fresh cover-slip preparations, in ten out of 316 examined by thin blood smears, and in eight out of 265 examined by thick blood films. Thiroux and D'Anfreville de la Salle (1911), and more recently Bourret (1913), have drawn attention to the fact that trypanosomes are seldom found by direct examination of a drop of blood in Senegal. The latter observer was successful by this method in only one case out of 45.

Thus the percentage of cases of sleeping sickness in which trypanosomes can be detected by an examination of the blood has been variously stated as from 45 % to 2 %. In the Eket district no case has yet been seen in which trypanosomes were to be found by an examination of a drop of the peripheral blood. Cases have been examined at all stages of the disease, and at different hours of the day, but always with negative results. Both fresh and stained preparations have been used, and thick dehaemoglobinized films have also been tried. Not only has it been our experience that trypanosomes could not be found in the blood by ordinary examination, but it was also that of Dr. Gray, who first identified the disease, and of Dr. Foran who spent a year in the district and collected a large number of cases.

Although blood examination for trypanosomes has been unavailing, the occurrence of auto-agglutination of the red blood corpuscles has been of some assistance in diagnosis. Yorke (1911) who studied this phenomenon concluded that 'a marked degree of auto-agglutination of the red blood cells is an extremely rare occurrence apart from an infection with trypanosomes.' Our observations were perforce made on coverslip preparations, a method of demonstrating the sign which is apparently fallacious. It may be of some interest to note, however, that in practically every case examined in this way auto-agglutination was well marked.

By gland examination

Since Greig and Gray (1904) stated that trypanosomes were almost invariably present in the glands of infected persons, gland puncture has assumed the first place in the routine examination for sleeping sickness. Dutton and Todd (1906) found trypanosomes in 97·2 % of cases by this method, and Koch and his colleagues (1909) in a similarly large percentage. Martin and Leboeuf (1908) were almost as successful, but they noted that as a rule the parasites were rather rare. Although therefore trypanosomes are not invariably to be found, there can be little doubt that in any case in which there was a sufficient enlargement of the cervical glands they would be explored by most observers before attempting any other method of diagnosis. In endemic areas in which the parasites are difficult to detect in the blood this method of examination is indeed the only convenient one to adopt.

All the cases diagnosed in the Eket district were examined in this way. In most of them a posterior cervical gland was punctured, but if no gland in this situation was sufficiently enlarged, one was chosen in the axilla or the epitrochlear region. In our experience trypanosomes were always rare in the gland juice, the heaviest infection met with during the months of November and December being seven parasites in thirty fields, and this was quite an exceptional case. It was often necessary to puncture a number of different glands before finding the parasites.

In many cases that for other reasons were considered highly suspicious of sleeping sickness gland puncture failed to reveal the presence of trypanosomes. In such cases excision of a cervical gland was resorted to. This practice was adopted not only because trypanosomes were sometimes found in the excised gland when they had been absent from gland juice obtained by punctures, but also because the patients were very willing to have this little operation performed, as it was the recognised native treatment for the condition.

Not infrequently, however, examination of the glands, no matter by what means, failed to reveal trypanosomes. More than a hundred cases of this sort have been met with. Clinically they suggested sleeping sickness, and the natives, whose acuteness of observation is remarkable, believed that they were suffering from

this disease. In some of them the glands were fibrosed, which may have accounted for the absence of the parasites. One of the cases proved fatal.

GLAND PALPATION

In Eket, since blood examination was fruitless, gland examination had to be relied upon for diagnosis. Centrifugalization of the blood or of the cerebro-spinal fluid could never be of much practical assistance except at headquarters owing to the skill and leisure required for its application; and inoculation into susceptible animals on a scale that would be essential to ensure success could not at present be contemplated. Gland palpation followed by gland puncture would therefore appear to be the most suitable measures to employ for the detection of cases, and for the determination of the areas affected by the disease.

It was not possible in the limited time at our disposal to make a systematic examination throughout the district, but the inhabitants of two selected villages were palpated for enlarged glands in the posterior cervical triangles. The results of these examinations are given below in tabular form (Tables 3 and 4). The first village, Ikot Offiong, was chosen because it was one of the places in which sleeping sickness was first observed, and is a typical example of a place in which the disease is prevalent. The second, Mpok, a town outside the area especially involved, was chosen to represent a village but slightly subjected to the attacks of the disease.

Ikot Offiong (Pl. XIX, fig. 1).

Ikot Offiong, a small village buried in dense bush, is situated near to Ikotobo, in the middle of the sleeping sickness area. It is some distance from the nearest stream, and is surrounded on all sides by trees and scrub. The village itself consists of a number of scattered compounds. Several cases of sleeping sickness have occurred here, and the village was considered to be a highly infected one. Of the 137 people examined, 53 were males and 84 females; the majority were children. Much enlarged (' + ') glands were present in 27·6 % of the boys, and in 17·5 % of the girls; and slightly enlarged (' + − ') glands in 48·3 % and 52·5 % respectively.

Too much reliance should not be placed on these figures since the number of persons examined was small. The most conspicuous feature was the number of children with glandular enlargements of the degrees ' + ' and ' + − .'

TABLE 3.—The incidence of enlarged posterior cervical glands among 137 natives at *Ikot Offiong* classified according to sex and age.

SEX	MALE			FEMALE		
Age	0—13	14—44	45—	0—11	12—39	40—
' + ' or much enlarged glands	27·6%	0·0%	0·0%	17·5%	0·0%	0·0%
' + — ' or slightly enlarged glands ...	48·3	0·0	0·0	52·5	20·8	20·0
' + — — ' or very slightly enlarged glands	17·2	22·2	16·7	20·0	25·0	20·0
' — ' or normal glands	6·9	77·8	83·3	10.0	54·2	60·0
Totals	100	100	100	100	100	100
Number of individuals of each class examined	29	18	6	40	24	20

Mpok

Mpok is a similar, but larger village. Unlike Ikot Offiong, it is situated to the west of the Qua Iboe River, in a locality where sleeping sickness is now almost unknown. Some years ago, indeed, a native 'doctor' lived in Mpok, and practised excision of glands for sleeping sickness; his patients, however, became so few that he was compelled to remove to another district. Altogether 825 persons were examined, 313 males and 502 females; of these 297 were children. Glandular enlargements of the degrees ' + ' and ' + − ' were much less common than at Ikot Offiong, and the

TABLE 4.—The incidence of enlarged posterior cervical glands among 825 natives at *Mpok* classified according to sex and age.

SEX	MALE			FEMALE		
Age	0—13	14—44	45—	0—11	12—39	40—
'+' or much enlarged glands	4·0%	0·0%	0·0%	1·7%	0·3%	2·8%
'+ —' or slightly enlarged glands ...	39·2	5·6	0·0	20·3	3·9	2·8
'+ — —' or very slightly enlarged glands	53·6	48·1	46·4	61·6	70·7	55·5
'—' or normal glands	3·2	46·3	53·6	16·3	25·0	38·9
Totals	100	100	100	99·9	99·9	100
Number of individuals of each class examined	125	160	28	172	304	36

TABLE 5.—Comparison of the degrees of enlargement of the posterior cervical glands among the children of Ikot Offiong and Mpok in the Eket district of Nigeria with those of the Gambia and the Congo.

AGE	MALES, 0—13				FEMALES, 0—11			
Place	Ikot Offiong	Mpok	Gambia, 1911	Congo, 1903-05	Ikot Offiong	Mpok	Gambia, 1911	Congo, 1903-05
'+' glands	27·6%	4·0%	0·4%	8·0%	17·5%	1·7	0·2%	6·2%
'+ —'	48·3	39·2	2·2	26·8	52·5	20·3	1·7	23·7
'+ — —'	17·2	53·6	42·0	22·9	20·0	61·6	28·0	22·4
'—'	6·9	3·2	55·4	42·3	10·0	16·3	70·1	47·7

children especially appeared to be more healthy. This fact, taken in conjunction with the prevalence of trypanosomiasis at Ikot Offiong and its rarity at Mpok, suggests that in the Eket district just as in the Congo and the Gambia there is a definite connexion between the occurrence of this disease and a high percentage of enlarged cervical glands.

In other parts of West Africa gland palpation has proved of the greatest assistance in the recognition of sleeping sickness. It is interesting, therefore, to compare the figures we have obtained in Eket with those recorded by the investigators in the Congo and the Gambia which have recently been summarized by Todd (1913). The condition in children is particularly significant (Table 5). With regard to considerable degrees (' + ' and ' + − ') of glandular enlargement, Todd writes 'There is certainly a close connection between these percentages of enlarged glands and the much more frequent occurrence of trypanosomiasis among natives of the Congo; it has already been made clear that, in the Congo as well as in the Gambia, enlarged cervical glands mean trypanosomiasis. In 1911, it was estimated that 0·8 % of the native population in the Gambia had trypanosomiasis; there, cases only occur sporadically. It is scarcely possible to estimate the probable percentage infected among the natives examined in the Congo in 1903-05, but it is certain that 50 %, and often more, of the natives in many of the villages visited were infected.' If enlargement of the cervical glands has a similar significance in Eket, and there seems little reason to suppose it has not, the incidence of trypanosomiasis must be great indeed, for within the bounds of a district of less than 700 square miles many thousands of cases must be congregated.

SYMPTOMS

The earliest record of sleeping sickness on the West Coast of Africa are unanimous in attributing a very heavy death rate to the disease (Atkins (1742), Winterbottom (1803), &c.). More recent investigations have proved, however, that in many parts the disease now occurs in a mild form. Dutton and Todd (1903) in the First Report of the Trypanosomiasis Expedition to Senegambia (1902) wrote 'Taking all our facts into consideration, we believe the

disease, as it occurs in natives, to be a peculiarly mild one, and that it is at present impossible to recognise it clinically,' and Horn (1910) in reporting on sleeping sickness in the Volta River district of the Gold Coast records that 'Although sleeping sickness is endemic in this part of the Gold Coast, and has been known to the natives for many years past, yet it is not at present in epidemic form, neither does it appear to have produced any epidemic within the memory of living natives, nor does it form any profound part of their traditions.'

The earlier writers may very likely have recognized only the advanced cases, but there is some evidence that trypanosomiasis in the course of years becomes less deadly in the endemic centres, although it appears to be capable of regaining its virulence when imported into new districts.

Mr. J. H. Harris (1912) in a recent book writes of sleeping sickness 'this scourge would seem to be spending its force. Seven years ago Uganda recorded over 8,000 deaths from sleeping sickness within twelve months, and the latest Government report shows that there has been a gradual reduction until in the year 1910 there were only 1,546. Happily this encouraging feature is present on the West Coast also. The Congo suffered more than any other colony . . . . Now, however, the absence of the scourge in many of the old districts is quite noticeable. Villages that we knew to be swept by this plague ten years ago are once more flourishing, and in some cases where the birth rate was almost nil the villages are again joyous with the laughter of little children.'

In Nigeria also sleeping sickness occurs in a mild form, apparently most similar to that in the Gambia. The disease in Eket, however, presents certain peculiarities which are difficult to explain adequately by a reduction in the pathogenicity of the parasite, the weeding out of the more susceptible individuals, or the establishment of a partial immunity.

The death rate from sleeping sickness in Eket seems to be very small. Of the 222 cases identified up to the end of 1913, nine had proved fatal, giving a mortality of 4 % for this period. Several of these patients had died outside the camp, so that it is not certain that in every case death was due to trypanosomiasis. For this reason, and because it is probable that it is the worst cases

that have hitherto come under observation, the figure given above is not likely to be too low a one. From other considerations also it might be expected that the mortality must be slight. The disease occurs especially amongst children, and if many of them died of it, one would expect to find a scarcity of adults, which is not the case. Eket is, indeed, one of the more densely populated districts of Nigeria.

The fact that the malady is of so mild a type does not necessarily minimize the gravity of the situation. Without further investigation it is not possible to forecast the results of its spread into neighbouring districts, where it might reassume the virulent form which, according to native tradition, it once had when it devastated whole towns. The position of Europeans and other non-natives must also be considered. Until the danger is proved not to exist, it would be wise for those responsible for the opening up of the country by roads and rail and for the recruiting of labour to remember the disastrous effects that have followed the introduction of sleeping sickness into other parts of Africa. It will be sufficient to recall the effect of removing the remnant of Emin Pasha's Sudanese soldiers into Uganda and Busoga to emphasize this point, and the notable instance recently given by Bernard (1913), who relates how sleeping sickness spread in all directions round Djema, where it had been first introduced with some labourers who had been brought to build a factory.

The symptoms exhibited by the patients in Eket were often of the slightest. A visitor to the isolation camp at Ikotobo, seeing the troops of children at play, or dancing on moonlight nights, would be struck by nothing so much as by the apparent good health, high spirits, and happiness of the inmates. Many of the patients indeed showed no signs of illness (Pl. XXII, fig. 16). Such symptoms as do occur are those common to trypanosomiasis all over Africa. It will therefore be unnecessary to describe them in detail, but the following brief notes will suffice to indicate the clinical aspect of the cases.

So many of the patients being children, it was impossible, in the majority of cases, to get a clear account of the onset of the disease. As a rule, glandular enlargement, of from one to five years' duration, was the only symptom complained of on

admission. In addition, fever, headache, and pains in the head and neck, were generally mentioned. The majority of the patients had been recognised to be suffering from trypanosomiasis (*Insip Itong*) for more than a year.

The difficulty experienced in confirming the diagnosis of trypanosomiasis in some cases may be explained by the long history of the infection, as a successful reaction on the part of the organism to the trypanosome is accompanied by sclerosis and disinfection of the glands, and the disappearance of the parasites from the peripheral blood. The former process, according to Thiroux and D'Anfreville (1909), precedes the latter by some months. Our experience, that gland puncture often revealed the presence of parasites whereas blood examination never did, is not necessarily in disagreement with this view, since we have been unable to prove the absolute sterility of the blood by examination after centrifugalization or by inoculation into susceptible animals.

With but very few exceptions the physical condition of the patients was good. The growth and development of the children did not appear to have been retarded, and the adults were for the most part well developed and muscular. In a minority of the cases some degree of emaciation was present, but in only one or two was it at all marked (Pl. XXI, fig. 7).

In a majority of the patients, on the other hand, the facial expression was somewhat dull. This feature, which was seldom pronounced in the children, was in part due to oedema of the face, and especially of the eyelids, which was a common sign (Pl. XXII, figs. 11 and 15). A few of the adult patients presented the typical dull, heavy faces associated with sleeping sickness. Prominence of the eyeballs was noted in three or four cases (Pl. XXII, fig. 14). The symptom does not appear to be a general or an important one, and is only mentioned because Vix (1912) observed exophthalmos in sixteen out of seventy cases examined in German East Africa.

Some degree of fever was invariably present, the highest point being reached in the afternoon, and the morning temperatures being normal or sub-normal. The fever was irregular in degree, but was seldom excessive. In very few cases was any sign of periodicity observed, such as that described by Kérandel (1910). As, however, most of the children were suffering from a concurrent

malarial infection (simple tertian, quartan, or most commonly malignant tertian), any such regular variation would probably have been masked or obliterated.

Disturbances of the alimentary system were almost entirely absent. The tongue was generally furred, but the appetite was unimpaired, and digestion appeared to be excellent. As usual in native subjects, an examination of the faeces revealed the presence of a rich and varied intestinal fauna. Ascaris, taenia, oxyuris and trichocephalus were all commonly found, and in addition, in a few cases, ankylostomes. In addition, trichomonas and a small spirochaete were frequently seen. The liver was slightly enlarged in about ten per cent. of the cases. Spasm of the pharynx was observed once. As in the case recorded by Thiroux and D'Anfreville de la Salle (1911) the condition was suggestive of rabies.

Enlargement of the lymphatic glands (Pl. XXII, fig. 12) was present in practically every case, and was not infrequently the only symptom observed. The natives themselves attached the greatest importance to this sign, and believed that by the complete excision of the glands the disease might be cured. As a rule, the condition was that of a polyadenitis. The glands varied greatly in size, and in the same individual both large soft glands and more or less indurated and fibrosed glands were often present. The glands in the parotid region were frequently involved, giving the patients a peculiar facial expression not unlike that associated with mumps (Pl. XXII, fig. 13).

As a rule the glands were painless, but in some cases they were said to be painful at certain times. Native belief associated the pain with the phases of the moon. When the moon was waning, it was said, the glands became tender and caused shooting pains to be felt in the neck and head. An adult infected with trypanosomiasis gave a very precise description of pain in the glands preceding their enlargement.

The spleen was almost always enlarged, a fact no doubt accounted for by the prevalence of malaria. Anaemia was also a common symptom, but was moderate in degree, the red cells seldom numbering less than 3,000,000 per c.mm.

The pulse was as a rule rapid, small, and of low tension.

Neither the respiratory system, nor the urinary system, appeared to be specifically affected.

Skin affections were generally present, and were of several types. Impetigo of the scalp was perhaps the commonest, but at the stage at which the patients came under observation was generally quiescent (Pl. XXI, fig. 10). The next most frequent condition was a papulo-pustular eruption distributed all over the trunk and limbs, but often most pronounced on the hands and knees. A scaly and very itchy erythema was also frequently seen, and a circinate urticaria was not uncommon.

Sexual symptoms, impotence and amenorrhoea, which are stated to be very early manifestations by Bernard, were met with in a few of the adult cases. In some instances menstrual disorders were the only complaints of the patients who came to the dispensary for treatment for these conditions, and were only discovered to be suffering from trypanosomiasis in the course of their examination. It is doubtful if impotence can be a common or pronounced symptom, for in every village visited children abounded, and, indeed, appeared to be unusually numerous.

Affections of the bones and joints were seldom observed, and those that were seen were probably rheumatic in origin. Rigidity of the flexor muscles of the left arm was present in one case. Cramps were never complained of.

Headache and neuralgic pains were commonly present. Serious involvement of the nervous system was, however, exceptional. Somnolence occurred in less than ten per cent. of the cases, and in very few indeed was there the tendency to fall asleep at inappropriate times (Pl. XXI, fig. 9). A lesser degree, characterised by listlessness and apathy, was more common. Speech was slow and deliberate in some of the patients who exhibited a tendency to somnolence. In one or two the gait was shuffling and uncertain. In one case a slight degree of unilateral facial paresis was present. The same patient, a boy of eight, had rigidity of the flexor muscles of the arm, and slight stiffness of the leg, on the same side of the body.

A fine tremor of the tongue was common. Coarse tremors of the limbs and tongue were present in advanced cases. The deep

reflexes were usually somewhat active. Romberg's symptom was sometimes noted. Deep hyperaesthesia was also observed occasionally. In one case epileptiform fits occurred. They came on suddenly one evening, and continued until about 2 a.m. the next morning. The next day the patient, although still somewhat dazed and stupid, seemed to have resumed her usual condition.

In the majority of cases intelligence appeared to be unimpaired, and several of the children were even so unusually acute as to be almost precocious.

TREATMENT

The routine treatment adopted at the camp has been a weekly intramuscular injection of 6 grs. of atoxyl. The patients appear to tolerate this dose, and up to the present no ill results have followed.

It would be difficult to judge of the action of any drug on a trypanosomiasis of the type found in Eket, for not only are the parasites undiscoverable in the blood and very rare in the gland juice, but also spontaneous cures frequently take place. The effects of treatment might, however, be estimated by the action on the temperature, and on the phenomenon of auto-agglutination of the red blood corpuscles.

As yet it is too early to give any definite opinion on the effects of atoxyl treatment at Ikotobo. In a few cases admitted to the camp in a greatly debilitated state the beneficial action has been striking, and the majority appear to have improved under treatment. Reduction in the size of the cervical glands, the sign that would appeal most strongly to the patients themselves and to their parents, has not yet been conspicuous. For the reasons given above it is impossible at present to determine to what extent the beneficial action of atoxyl has been due in its effect on the trypanosomes, and to what extent to its tonic properties.

Intercurrent infections, as, for instance, malaria, have of course been treated also at the camp. The general improvement in health attained in this manner may account in part for the good results observed.

THE PATHOGENIC AGENT

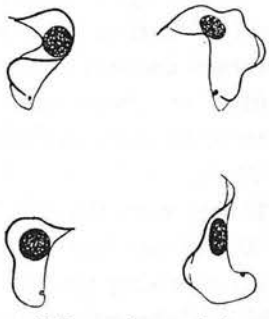
In his 'General Report for the year 1912,' Dr. Foran wrote with regard to the parasite he had found in gland juice from the cases of sleeping sickness in Eket: 'Judging from measurements and general characteristics, I believe this parasite to be the well-known Trypanosoma Gambiense.' In a former paper, however, one of us (Macfie, 1913) described the morphology of the trypanosome as it appeared in the blood of a guinea-pig that had been infected by inoculation with cerebro-spinal fluid from one of the cases under observation at Ikotobo, and as it presented certain peculiarities, proposed for it the name *T. nigeriense*. The virulence of the parasite to the smaller laboratory animals has been found to be extremely low, a fact which has been considered to support the contention that it is not the same species as *T. gambiense*.

During the present investigations, further evidence has been sought on the nature of the pathogenic agent. As already pointed out, the parasites were only found in the gland juice, and in very small numbers. The forms encountered were similar to those previously described, and in one instance a very small individual of the type believed by us to be characteristic of *T. nigeriense* was found.

Guinea-pigs and white rats were the only animals available for experimental purposes. White rats had already been proved to be refractory, and it was not surprising that all attempts (4) to infect them proved unsuccessful. Three out of twelve guinea-pigs were infected by inoculation with blood containing trypanosomes of the strain obtained from Eket earlier in 1913, and it was anticipated that it would be possible to infect this species of animal from other cases of sleeping sickness. Seven guinea-pigs were inoculated with gland juice known to contain trypanosomes from as many cases of human trypanosomiasis, but only one became infected. A guinea-pig was also injected intraperitoneally with 5 c.cm. of blood from a proved case of sleeping sickness, but failed to become infected. A single wild mouse (*Mus musculus*) was inoculated, with a negative result.

The single guinea-pig that became infected had been inoculated subcutaneously with gland juice from one of the cases of sleeping sickness admitted to the camp in December, an adult male. The

animal had been under close observation for seven weeks before inoculation, and had been kept in a double-fronted mosquito-proof cage. The trypanosomes first appeared in the blood in sufficient numbers to be detected on the twenty-seventh day. For several days (4) before this their presence had, however, been suspected, owing to the occurrence of well-marked auto-agglutination of the red cells. The parasites were at first extremely rare. On each of the first five days of the infection ten trypanosomes were found with difficulty, and were measured (Table 6). The longest of these fifty individuals measured 24 μ, the shortest 13 μ, and the average length was 17·54 μ. In the course of a day or two, however, the trypanosomes became rather less rare, although still far from numerous, and both longer and shorter forms were found. Some of the latter were of the type which we believe to be characteristic of this strain (see Text-figs).



× 2000. *T. nigeriense*, small forms characteristic of the strain, for comparison and contrast with the atypical stumpy forms of other polymorphic trypanosomes occasionally found in guinea-pigs (see Pl. XXIII, figs. 4, 5 and 6).

The extreme rarity or complete absence of trypanosomes from the peripheral blood of all the human cases examined, the rarity of the parasites even in the gland juice, and the difficulty experienced in infecting animals by inoculation, suggest that it must be a very exceptional occurrence for a tsetse fly to become infected by feeding on these cases. It has been estimated by one of us (1912) that the average amount of blood taken up by *G. palpalis* in an uninterrupted feed is about 3 c.mm. If, therefore, one of these flies fed fully on an infected human being every day, it would be over four years before it had ingested 5 c.cm. of blood, an amount which, in the experiment referred to above,

failed to infect a guinea-pig when inoculated into its peritoneal cavity!

It is possible, of course, that at some stage of the disease trypanosomes may be present in the blood in considerable numbers. It is also possible that the blood may contain elements capable of infecting tsetse flies, but incapable of infecting vertebrates. Both these hypotheses are unsupported by any evidence, and are at least improbable. It seems, therefore, that the human infections must be dependent on some other cycle of development, including an

TABLE 6.—Measurements of the human trypanosome during the first five days of an infection in a guinea-pig.

Day of the infection	Number measured	Length in Microns		
		Average	Minimum	Maximum
1	10	18·9	16	24
2	10	17·8	13	24
3	10	17·1	14	21
4	10	17·5	14	22
5	10	16·4	14	19
	50	17·54	13	24

insect and some so far unidentified animal host, the reservoir of the disease. The ordinary development of the trypanosome may take place in these two hosts. The insect, infected from the animal host may, however, be capable of infecting human beings; but the disease may be so modified in them that they are incapable of handing on the infection any further. In this connexion the results obtained by Bruce and his collaborators (1913, 1) in the case of *T. simiae* are of interest. They found that monkeys were readily infected with this trypanosome by the bites of *G. morsitans*, but that inoculations with blood of goats containing the same

parasites were usually unsuccessful, the passage of the trypanosome through the body of the goats having reduced its virulence for monkeys almost to nothing.

A similar reduction of the virulence may take place in the case of the human trypanosome in its passage through the bodies of the natives of Eket. The question then arises whether the unusual symptomology, and the atypical morphology and pathogenicity of the parasite, may not be accounted for by the modifying influence of the human host, and whether it is necessary to suppose that *T. nigeriense* and *T. gambiense* are specifically different. It is recognized nowadays that, in the words of Bruce and his colleagues (1913, 2): 'It is absurd to expect to arrive at any classification at all approaching a true one by the study of strains of trypanosomes kept for many years and undergoing many vicissitudes in our European laboratories,' but although the pathogenicity may undoubtedly vary, we think it has yet to be proved that the morphology may undergo definite changes, except as the result of the action of certain drugs. Such, at any rate, appears to be the significance of the experiments of Blacklock and Yorke (1913) with *T. vivax*. These investigators succeeded in modifying the virulence of this parasite so that it became pathogenic to rabbits, but after passage through six generations of these animals they found that 'the trypanosomes appeared to be identical with those seen in goat's blood.' While admitting the possibility, which in the interests of simplicity would be very readily entertained by us, we find ourselves unable at present to convince ourselves that the strain of human trypanosome found in Eket is identical with *T. gambiense*.

THE INVERTEBRATE HOST

The rarity of *G. palpalis* in the parts of the Eket district especially affected by sleeping sickness has already been mentioned. It is difficult to understand how so small a number of these insects can be responsible for so large a number of cases, especially when the absence of the parasites from the peripheral blood of the human patients is taken into consideration. On the eastern side of the district, *G. palpalis* is certainly more common, but in this part of

the country sleeping sickness is much less frequent. It is impossible, however, to exclude *G. palpalis* as a factor in the transmission of the trypanosomiasis, for in every place which has been carefully and systematically searched it has been found, although, perhaps, with extreme rarity.

G. tachinoides, on the other hand, is much more abundant, and is particularly associated with the parts of the district in which sleeping sickness is most prevalent. A number of experiments were carried out to determine with what trypanosomes these flies were naturally infected. A full account of them is given below (Appendix 2), but it may be stated here that wild flies were found to be capable of transmitting *T. pecaudi* (*T. brucei*, of Uganda), *T. vivax*, and *T. pecorum*. Owing to the relative insusceptibility of the experimental animals employed to the Nigerian strain of human trypanosome, these experiments cannot be regarded as excluding the possibility that the wild *G. tachinoides* may also be infected with this parasite. Indeed, since they are capable of transmitting the three trypanosomes recorded above, it seems not improbable that they may also transmit *T. nigeriense*. Assuming that the disease is transmitted by a tsetse fly, it seems more probable that it will prove to be by *G. tachinoides* than *G. palpalis*.

It is now well known that several pathogenic trypanosomes, including *T. gambiense*, can be transmitted by more than one species of tsetse fly; and the conclusion to be drawn from the work that has been done on this subject would seem to be that they may develop in any species of *Glossina*. It still remains uncertain whether particular tsetse flies are specially adapted for the development of certain trypanosomes. In the case of *T. gambiense* development has generally been supposed to take place in *G. palpalis*, although Kleine and Fischer have found it occur in *G. morsitans* also. It seems reasonable to suppose, therefore, that *G. tachinoides* may also act as the invertebrate host. Whether such an association would be attended by any modification of the virulence of the parasite, or by such morphological changes as those described by us in the case of *T. nigeriense*, is at present unknown.

The members of the French Commission, impressed by the

absence of parallelism between the frequency of occurrence of *G. palpalis* and that of cases of sleeping sickness, and having encountered many instances in which several members of a family living in the same hut were infected while other natives of the same village were not attacked, concluded that the agent of infection must be an insect biting in the night. *G. palpalis*, they stated, was the principal agent of infection. Its presence was absolutely necessary for the propagation of the disease to a distance, and for the maintenance of endemicity; but in addition, they thought, mosquitos and probably other biting insects might be important auxiliaries.

A similar lack of parallelism between tsetse flies and sleeping sickness seems to exist in Eket. In several instances also two or three members of a family were found to be infected, and the natives appear to believe that the disease may run in families. For these reasons it was thought possible that some other insect might play a part in the dissemination of the disease.

It has already been mentioned that impetigo of the scalp was a frequent and early symptom in our cases of trypanosomiasis. In several instances this condition of the skin was clearly stated to have preceded the onset of the disease. Why this impetigo should occur at all it is difficult to understand, as it is not, we believe, a common disease of native children. On one occasion it was suggested that it had originated in the bites of some insects, and as bugs (*Cimex lectularius*) are very common parasites of the natives, it was considered of interest to examine these insects with a view to determining if they were capable of transmitting trypanosomiasis.

Twenty-three bugs, collected from the beds of sleeping sickness patients, were therefore fed on a guinea-pig for eighteen days. At the end of this period only two of the bugs remained alive. The guinea-pig was kept under observation for forty-three days, but trypanosomes were never found in its blood, neither was auto-agglutination of the red cells noted. But little reliance can be placed on the result of this single experiment, especially as guinea-pigs are not highly susceptible to the human trypanosome.

THE ALTERNATIVE VERTEBRATE HOST OR RESERVOIR

Unfortunately it was impossible during the short time at our disposal to determine the reservoir of sleeping sickness in Eket. Neither the necessary material, nor the requisite experimental animals, for the purpose could be obtained, and the subject will have to remain for some future investigation.

With regard to game, the country is too well populated to harbour much, and, in fact, it is rarely met with in the greater part of the district; and what there is, unless it is assumed that the great majority is infected, seems insufficient to account for the number of human beings found to be suffering from trypanosomiasis. We were unable to procure a single antelope for examination, although we offered to the natives a substantial reward for every animal they would bring into the camp.

In the few examinations it was possible to make we failed to implicate domestic animals. The natives of Eket are still somewhat shy of Europeans, and were reluctant to allow their domestic animals to be examined. In almost every village, sheep, goats, dogs, dwarf cattle, and occasionally pigs were found. Trypanosomiasis was found to occur in these animals, and the dwarf cattle were infected with a minute babesia, probably *Theileria parva*, which, however, did not appear to cause any symptoms of illness. It would be interesting to know if this infection is in any way connected with the apparent immunity of these cattle to trypanosomiasis.

Altogether sixteen animals were examined, namely, four sheep, four goats, two dwarf cattle, four dogs, and two pigs; and of these four were found to harbour trypanosomes. *T. vivax* was found in one sheep and one dwarf cow, and a trypanosome of the type of *T. pecorum* in one pig and one sheep. All four animals appeared to be healthy. From the feeding experiments with wild *G. tachinoides* it is evident that *T. pecaudi* (*T. brucei*, of Uganda), *T. vivax*, and *T. pecorum*, at any rate, must be common in the district, either in the domestic or wild animals, or in both. No domestic animal was found to harbour trypanosomes of the human type.

In a recent discussion on the relation of big game to sleeping sickness, it was suggested that there might be human 'carriers' of trypanosomiasis. Prof. Yorke (1913), who opened the discussion,

stated that in the case of *T. rhodesiense* 'The hypothesis that human beings can harbour the parasites for long periods without detriment to health, and thus act as reservoirs of the virus in the same way as the game,' was one which he could not support. In the case of a parasite with but a slight degree of pathogenicity, however, the possibility of there being human 'reservoirs' is greater, a view apparently held by Dutton and Todd (1903) when they wrote of *T. gambiense* in Senegambia: 'The slight virulence of this trypanosome, and the consequent lack of symptoms, has frequently suggested to us the possibility that the natives in this disease may bear the same relation to the Europeans as does the wild game of Central Africa to domestic animals in the tsetse fly disease.'

Bearing in mind the number of infected persons and the chronicity of the disease in Eket, it might be thought that in this district human beings might act as the reservoir. The fact that trypanosomes have not been found in the peripheral blood in a single case is undoubtedly a strong argument against this hypothesis. But as we have not been able to centrifugalize blood it must be remembered that the parasites may actually be present, but in such small numbers as to escape detection in an ordinary examination.

SUMMARY AND CONCLUSIONS

There is in the Eket district of Nigeria an endemic focus of sleeping sickness of considerable magnitude, and it is probable that a large percentage of the poulation either is or has been infected with the disease.

The disease is of a mild type, the mortality is low, and spontaneous cures appear to be frequent. The patients are mostly children.

Although trypanosomes can be found in the gland juice, they have never yet been detected in the peripheral blood. These trypanosomes differ in some respects in their morphology and their pathogenicity from typical strains of *T. gambiense*, and we believe that the two parasites are not of the same species.

The species of insect that transmits the disease in Eket to man, and the animal that acts as the reservoir of the virus, have not yet been determined. There is some reason to suspect that *Glossina tachinoides* may be found to carry the infection.

APPENDIX I

NOTES AND OBSERVATIONS ON SLEEPING SICKNESS IN THE EKET DISTRICT

BY

W. C. W. EAKIN

History. In the beginning of 1906 a native of Ikotobo came to the Mission Dispensary asking for medicine for sleeping. He mentioned that the disease was known to the natives as *Odono Idap*, or Sleeping Sickness. The writer thought it advisable to go and see the patient, and found a young adult woman lying under the veranda of a native hut, apparently dying. The husband stated she had been ill for over a year with a sleeping sickness, and was incapable of taking care of herself, as on one occasion she fell asleep while cooking food, fell into the fire, and was badly burned. The woman lingered for a short time and died a month later.

The suspicious nature of this case with its attendant symptoms raised the interesting question as to whether the sleeping sickness known in other parts of Africa had made its way to Southern Nigeria.

Again, in May, 1911, a chief from the town of Ikot Offiong, in the Oron part of the district, brought his daughter, a girl of about sixteen years of age, for medicine. The father stated she suffered from pains in the head followed by sleeping, and was unable to do her work. The girl appeared very ill, and the glands of her neck were abnormally enlarged. She slept whilst the chief described her case. He pointed to the glands, and asked if they could be cut out, as he believed their excision would effect a cure. He also mentioned that his eldest daughter had died the previous year with the same disease. Nothing could be done for her, and it was reported that she died a month later.

From recent information it appears the father of the girl also died of sleeping sickness, making a death roll in that family of three persons inside the last three years.

It was thought that these facts should be reported without delay, and Mr. S. A. Bill, of the Qua Iboe Mission, from informa-

tion supplied by the writer, forwarded them to the Medical Officer, Calabar. Action was taken in due course, and Dr. R. W. Gray visited the district to investigate the matter. He established the diagnosis of sleeping sickness by finding the trypanosomes. He was followed by the late Dr. P. F. Foran to continue the investigation, and adopt measures to check the spread of the disease. At his request the following notes were obtained from chiefs and others resident in the district, and they may be taken to represent the native point of view, without modification by the writer, as to the history of sleeping sickness, its origin, infectious nature, and the measures adopted to prevent it spreading, and it is hoped they may throw some light on the matter.

Prevalence. Sleeping sickness is prevalent throughout the district with the possible exception of Ibuno—where a small tribe of Ibunos has settled at the mouth of the Qua Iboe River—and the natives assert that it is widely distributed.

Names. Two stages are known and recognised. The first characterised by gland enlargement called *Insip Itong*, and the second or somnolent stage as *Odono Idáp* (pronounced Awdongaw Edap).

Tradition. While too much credence cannot be given to statements made by natives, yet the majority of those interviewed affirmed that:—It is generally believed that the town of Ikotobo, very many years ago, was nearly depopulated by an epidemic of sleeping sickness. They state the disease is less fatal now owing to successful treatment by the native doctors in removing the enlarged glands. A strong feeling has grown up among chiefs and old people that it is now greatly on the increase, and especially in the towns and villages situated on the banks of the Obium River. The reasons for holding this opinion are that:—

1st. The laws controlling the habits of those suffering from *Insip Itong* are now disregarded.

2nd. Isolation is no longer considered necessary.

Cause. Information has been sought for from many sources as to the cause of sleeping sickness. The natives believe it can be contracted in any of the following ways:—

1st. By the shooting of a flaming or burning arrow from the gun or bow of a witch doctor.

2nd. By coming in contact with an infected person, such as eating with his spoon, wearing his cloth, or sleeping on his bed.

3rd. By a strange native laying his hands on the head of any person and invoking the evil spirits to cause the sickness.

In addition to the reasons given above, an exception was found in Ikotobo, where some natives attributed sleeping sickness to the bite of a fly known as the *Nsung Idap*, or sleeping fly. This insect is commonly known to every town in the district as the *Nsung Obio* or town fly. This fly has been identified as a tsetse fly.

Laws and Customs. An ancient code of laws and customs is in existence for controlling the habits of those suffering from *Insip Itong* and sleeping sickness. The general belief is that both stages are infectious, and that isolation is necessary to prevent it spreading.

The code is composed as follows:—

(*a*) Persons suffering from *Insip Itong* must provide their own spoon, plate, and cooking pot.

(*b*) They are forbidden to:—

1st. Wash in the public watering-places.

2nd. Use the cloth of another person.

3rd. Sleep on a bed or use a mat of another.

4th. Eat rice or any food of a granulous nature.

(*c*) If infected persons visit a native house they must not sit on a mat or seat, nor eat with a spoon or use any utensil. They must sit or sleep on the ground, eat with their fingers, using plantain leaves for plates.

(*d*) All persons in the *Insip Itong* stage are considered unclean until the glands are removed by a native doctor.

(*e*) When the glands are removed notice is given to the chiefs, and a day is appointed for the ceremony of receiving back to society persons pronounced cured by the native doctor. The whole town is called to the public meeting-place where it is to take place. The ceremony begins by presenting the cured persons to the chiefs. This is performed by the native doctor. The clothes they wore and the beds, with the utensils they used, are at this

stage brought and burned publicly. A sacrifice of a fowl is made to the spirits by the doctor, who next proceeds to tie round the neck of his late patients a charm consisting of a shell enclosing a palm kernel. This is worn as a sign that the disease has been cured. A feast is then prepared in their honour, and the day ends up with dancing and singing.

Particulars. It is stated that the disease is equally distributed over males and females, and that the majority of those infected are children and young adults. If adults contract it the prevailing belief is that they cannot be cured.

Occupation. Natives of the trading, but more particularly of the farming, class, suffer most from sleeping sickness.

The belief is held that it can run in families, and cases have been cited where the father died, followed by his son and other members of his house.

It cannot be stated definitely that any particular place is associated with the disease, but particular towns and villages are known by the natives to suffer more than others.

Symptoms and Course. The first symptoms and the order in which they come in the case of children are given as follows:—

1st. Gland enlargement.
2nd. Shooting pains in the head and neck.
3rd. Fever and night sweats.
4th. Rash covering the body.

If the glands are not removed a second stage is reached, when the patient becomes weak, subject to fits, somnolence, and death.

The course of the disease is more rapid in the case of adults, and the periods between the *Insip Itong* and sleeping stages may vary from six months to one or two years before death.

In children the first stage runs on for years before the second is reached. It is difficult to obtain reliable information from the natives as to the length of time from the appearance of the first symptoms till the second or sleeping stage was reached, and from that till death intervened. This is due to their inability to keep count for longer than one year. The case of the girl of sixteen mentioned in the beginning of these notes was brought to the Mission Dispensary in May, sleeping. She had reached the

sleeping stage six months previously, and died the following month. No information was obtained as to how long she was in the *Insip Itong* stage.

While persons suffering from sleeping sickness become weak and emaciated, it has been noticed they are able to eat as well as when in health. Fertility is not diminished, but according to custom, a man having a wife suffering from *Insip Itong* must have the glands removed without delay.

Mortality is said to be greater among adults than children.

Treatment. *Insip Itong* doctors are found in many towns, and one town called Odoro Atabong has no less than seven. Their business is to excise the glands, from the neck, axilla, and groin. Their fees vary according to the wealth and position of the patient. It is almost impossible to calculate the exact amount they demand for their services, but it may be assumed that £5 is the lowest sum they are willing to take for the operation. The 'cure' may extend over two or three years, and consist of several operations. Their methods and surgical instruments are of a primitive nature. Guarantees must be given before they are willing to operate, and it is usual for a substantial instalment to be paid over when the first operation is complete.

A native doctor proceeds as follows:—

A small piece of bush having been cleared the patient is brought to the spot in the middle of the night by the doctor, accompanied by his parents or friends. A place is prepared for him to sit, and he is warned not to move. A fire is kindled and some burning faggots taken from it by the doctor and whirled round the head of the patient while some formula, invoking the spirit to make the sickness obedient to the knife of the operator, is used.

This ceremony finished, the patient is led home, his head shaved, and some root medicine rubbed into the scalp. This medicine has the effect of making the skin peel off.

The person is next tied hand and foot to the veranda post of the house and the operation begins. It consists of cutting out a number of glands from the neck and other parts of the body. Six or seven glands may be excised at the first operation.

It is stated that many persons recover, but it is not known how many die after the operation.

Diseases in Animals. There was a time when droves of pigs were kept in large numbers, but by an ancient law they were destroyed.

The natives believe that pigs are:—

1st. Unclean and bring sickness.

2nd. Increase the number of *Nsung Obio* or town fly.

3rd. Responsible for many skin diseases.

Inquiries have been made to find out if there was any connection between this law of prohibition and sleeping sickness, but without result.

These notes would be incomplete without making special reference to the work of the late Dr. P .F. Foran during the twelve months he spent in the district. He displayed marked ability, and the success he attained demands the highest praise. He was entrusted with the difficult tasks of obtaining information from native sources as to the history, cause, &c., of sleeping sickness, and of establishing an Isolation Camp for infected persons. These he undertook with unflagging energy and unwearying patience. Before he left he was rewarded with the confidence of the chiefs and people, and as a result of their co-operation infected persons began to pour into the Isolation Camp he had prepared for them. He was untiring in his efforts to make the camp a congenial place for the patients. A school was commenced, and knitting, basket making, &c., instituted, in order that the patients might be happy and contented in their new surroundings. No adequate tribute can be paid to the brilliant and hard work of this officer. He encountered initial difficulties from prejudice and opposition that would have defeated most men, but his keen attention to every detail for the welfare of his patients, coupled with his kindly disposition, won for him an esteem that will live long in the memories of those with whom he came in contact.

W. C. W. EAKIN.

IKOTOBO,

November 24th, 1913.

APPENDIX II

TRYPANOSOMES FOUND INFECTING WILD *GLOSSINA TACHINOIDES*

BY

J. W. SCOTT MACFIE, M.A., B.Sc.

Six experiments were carried out at Ikotobo in November and December, 1913, with the object of determining the species of trypanosomes transmitted, in nature, by *G. tachinoides*. In four cases the flies were fed on clean guinea-pigs; in one case on a clean rat; and in one case on a clean goat. All the animals were kept in double-fronted mosquito-proof cages to obviate the danger of accidental infections; and in order to exclude the possibility of mechanical transmission, the flies were starved for two days after capture before being given their first experimental feed. Unfortunately no monkeys were available for experiment, so that it was very unlikely that any evidence of infection with the human trypanosome could have been looked for considering the relative insusceptibility to this strain of the species of animal employed.

Altogether 145 flies were used. The details of the experiments are given below, and the general results are shown in the subjoined table (Table 7). Five of the six animals developed trypanosomiasis, so that at least five out of the 145 flies must have been infected.

TABLE 7.—Results of feeding wild *G. tachinoides* on uninfected animals at Ikotobo.

Experiment No.	Number of flies fed	Animal	Result
1	9	Guinea-pig	Negative
2	37	,,	*T. pecaudi* (*T. brucei*, Uganda)
3	25	,,	*T. pecaudi* (*T. brucei*, Uganda)
4	16	,,	*T. pecorum*
5	22	White Rat	*T. pecorum*
6	36	Goat	*T. vivax*

Three species of trypanosome were thus transmitted, namely, *T. pecaudi* (*T. brucei*, Uganda type) to two guinea-pigs, *T. pecorum* (*T. congolense*) to a guinea-pig and a white rat, and *T. vivax* to a goat.

Transmission experiments have been carried out by a large number of workers, but so far as I am aware *G. tachinoides* has not previously been proved to transmit in nature either *T. pecaudi* (*T. ugandae*), or *T. vivax*. The experiments described here, therefore, lend further support to the belief that pathogenic trypanosomes may, under suitable conditions, develop in several species of tsetse fly.

TABLE 8.—Proportions of wild *G. tachinoides* infected with trypanosomes.

Species of Trypanosome	Number of flies fed	Number of infections	Ratio of infected to uninfected flies
T. pecaudi (*T. brucei*)	145	2	1 : 72
T. pecorum	145	2	1 : 72
T. vivax	36	1	1 : 36

The percentage of *G. tachinoides* infected in the Eket district was extremely high. In the case of *T. pecaudi* and *T. pecorum*, to which all the three species of animal were susceptible, at least two out of the 145 flies must have been infected with each, giving a ratio of infected to not-infected flies of 1 to 72. In the case of *T. vivax*, to which guinea-pigs and rats are refractory, at least one out of thirty-six flies must have been infected. It is possible that, employing such a small number of flies, good fortune played a part, and that the ratios might not have worked out so high had a larger number of tsetse flies been available.

Although such a high percentage of the tsetse flies was found to be infected with a trypanosome characterized by posterior nuclear forms (*T. pecaudi*, *T. brucei* of Uganda), no human infection with this type of parasite (*T. rhodesiense*) has as yet been identified.

TABLE 9.—Animal reactions of the trypanosomes isolated from *G. tachinoides* at Ikotobo.

Strain of trypanosome	Source of the virus	Incubation period	Duration	Remarks
T. pecaudi (*T. ugandae*).—				
Guinea-pig No. 5 ...	*G. tachinoides* ...	?	?	Died on the 32nd day after the first feed, and the 11th day after the last feed, of the flies.
Guinea-pig No. 9 ...	*G. tachinoides* ...	7	33	
Rat No. 16	Guinea-pig No. 5	7	18	
Rat No. 23	Guinea-pig No. 9	5	19	
T. pecorum (*T. congolense*)—				
Guinea-pig No. 14 ...	*G. tachinoides* ...	?	42	Died on the 42nd day after the first feed, and the 39th day after the last feed, of the flies.
Guinea-pig No. 29 ...	Guinea-pig No. 14	9	47	
Rat No. 19	Guinea-pig No. 14	—	—	Did not become infected
Ram No. 28	Guinea-pig No. 14	7	57+	Alive and well on the 57th day
Guinea-pig No. 15*b* ...	Ram No 28 ...	14	42+	Still alive on the 42nd day
Rat No. 7	*G. tachinoides* ...	?	107+	Flies fed on days 1-5. Alive and well on the 107th day
Rat No. 26	Rat No. 7 ...	—	—	Did not become infected
Guinea-pig No. 15*a* ...	Rat No. 7 ...	—	—	Did not become infected
Guinea-pig No. 372 ...	Rat No. 7 ...	—	—	Did not become infected
Dog No. 370	Rat No. 7 ...	—	—	Did not become infected
Dog No. 371	Rat No. 7 ...	—	—	Did not become infected
T. vivax—				
Goat No. 21	*G. tachinoides* ...	?	81+	Flies fed on the first 14 days. Alive and well on the 81st day, but still infected.
Guinea-pig	Goat No. 21 ...	—	—	Did not become infected
Rat	Goat No. 21 ...	—	—	Did not become infected

T. pecaudi (*T. brucei*) (Pl. XXIII, figs. 1 to 10).

In two experiments, infections with a polymorphic trypanosome resembling *T. pecaudi* or *T. brucei* (Uganda type, *T. ugandae*) were obtained by feeding wild *G. tachinoides* on guinea-pigs.

EXPERIMENT 2

In the first experiment the flies, after a preliminary period of starvation lasting two days, were fed on guinea-pig No. 5 as they were brought in by the collector. In this way altogether thirty-seven flies fed on the animal between November 21st and December 12th. On the latter date the guinea-pig was found to be infected, and the feeding of the flies was stopped. In this instance, therefore, it is not possible to state what was the incubation period.

The trypanosomes were fairly numerous at first, but became extremely scanty from the 4th to the 9th day after their first appearance in the blood. On the 10th day they were again fairly numerous, and on the 11th day (December 22nd) the guinea-pig died.

Morphologically the trypanosomes were somewhat unlike the forms of *T. pecaudi* (*T. ugandae*) usually seen in a guinea-pig. The stumpy individuals were smaller and rather more slender (Pl. XXIII, figs. 4-6), and forms with posterior nuclei were never found. Twenty trypanosomes were measured on each of the four days on which it was possible to find this number in the blood films taken (Table 10). The longest of these eighty individuals measured 35μ, the shortest 14μ, and the average length was 25·87μ.

A single white rat, No. 16, was inoculated from guinea-pig No. 5, and developed trypanosomes typical of *T. pecaudi* (*T. ugandae*). Posterior nuclear forms were abundant during the latter days of the infection, when the parasites were present in the blood in enormous numbers (Pl. XXIII, figs. 7-10). The incubation period in this rat was seven days, and death occurred on the 18th day.

EXPERIMENT 3

In the second experiment twenty-five starved *G. tachinoides* were fed once only, on December 13th, on guinea-pig No. 9. On the 7th day trypanosomes first appeared in the guinea-pig's blood, and on the 33rd day death occurred. A white rat inoculated with

the blood of this animal on the 20th day became infected, the incubation period being five days, and the duration of the disease nineteen days. In both these animals the trypanosomes resembled typical specimens of *T. pecaudi* (*T. ugandae*) and posterior nuclear forms were found.

The trypanosomes isolated in these two experiments were definitely polymorphic, and morphologically identical with either *T. pecaudi* or *T. brucei* (*T. ugandae*). These two species of trypanosome would appear to be indistinguishable, and are probably the same organism. Posterior nuclear forms were found in both strains. Of 280 individuals measured the longest was 35μ, the shortest 14μ, and the average length was 24·78μ (see Table 10). The incubation period, and the duration of the disease, in guinea-pigs and white rats is compatible with the identification given above.

TABLE 10.—Measurements of *T. pecaudi* (*T. brucei* of Uganda).

Host	Day of the infection	Number measured	Length in Microns		
			Average	Minimum	Maximum
Guinea-pig 5	1	20	26·6	20	32
,,	2	20	26·0	17	34
,,	3	20	28·5	17	35
,,	10	20	22·4	14	32
Guinea-pig 9	4	20	28·75	24	34
,,	6	20	22·95	14	32
,,	8	20	21·1	17	29
,,	10	20	23·7	18	32
,,	12	20	27·85	22	32
Rat 16	2	20	24·7	20	28
,,	4	20	24·1	21	35
,,	6	20	22·25	18	32
,,	8	20	23·75	18	33
,,	10	20	24·4	18	32
		280	24·78	14	35

TABLE 11.—Distribution, by percentages, in respect of length of *T. pecaudi* (*T. brucei* of Uganda).

Host	Number measured	Distribution, by percentages, of lengths in microns										
		14	15	16	17	18	19	20	21	22	23	24
Guinea-pig 5	80	1	...	1	4	4	1	7	3	1	7	9
Guinea-pig 9	100	1	...	...	2	9	8	7	4	5	1	8
White Rat 16	100	...	...	...	...	3	2	6	14	12	16	12
Totals, to the nearest whole numbers ...		1	0	0	2	5	4	7	7	6	8	10

Host	Number measured	Distribution, by percentages, of lengths in microns										
		25	26	27	28	29	30	31	32	33	34	35
Guinea-pig 5	80	6	3	6	8	10	15	6	4	...	3	1
Guinea-pig 9	100	6	5	7	10	7	8	4	5	2	1	...
White Rat 16	100	11	9	4	4	1	...	1	2	2	...	1
Totals, to the nearest whole numbers ...		7	6	6	7	6	7	4	4	1	1	1

T. pecorum (*T. congolense*) (Pl. XXIII, figs. 15 to 31).

In one guinea-pig, and one white rat, on which wild *G. tachinoides* had been fed, infections were obtained with small trypanosomes of the *congolense-pecorum* type.

EXPERIMENT 4

In this experiment sixteen starved *G. tachinoides* were fed for three days, December 16-18, on guinea-pig No. 14. Up to the 23rd day no infection had been noted, and from the 24th to the 31st day it was impossible to make any examinations. On the 32nd day, however, a few trypanosomes were found in the guinea-pig's blood, and on the 42nd day the animal died. On the 38th day a ram, a guinea-pig, and a white rat were subinoculated from guinea-pig No. 14. The rat did not become infected. The ram

showed trypanosomes for the first time on the 7th day, and the guinea-pig on the 9th day. In both animals the parasites were at first extremely rare, and might easily have been overlooked had not a prolonged and careful search been made for them.

The infection in the ram remained slight up to the 57th day, when the experiment was concluded. The trypanosomes had never been otherwise than scarce, and had on several occasions been absent from the blood films examined. The animal had never shown any symptoms of illness. A guinea-pig subinoculated with the blood of this ram first showed trypanosomes on the 14th day.

In the guinea-pig No. 29, on the other hand, the trypanosomes steadily increased in numbers as the disease advanced. On the 45th day the animal seemed to be somewhat unwell, and on the 47th day it died.

TABLE 12.—Measurements of *T. pecorum* (*T. congolense*).

Host	Day of the infection	Number measured	Lengths in Microns		
			Average	Minimum	Maximum
Guinea-pig No. 14 ...	1	25	13·36	11	16
,,	6	25	13·08	10	18
,,	8	25	13·92	10	18
,,	9	25	13·12	9	16
Ram No. 28	16	20	15·20	12	18
,,	18	20	14·35	12	17
,,	20	20	14·50	10	18
,,	21	20	14·25	12	17
Guinea-pig No. 29 ...	18	20	14·25	12	17
,,	19	20	12·75	10	16
,,	20	20	14·90	12	17
Rat No. 7	1	10	15·9	13	21
,,	5	10	14·4	11	18
,,	6	10	15·7	12	20
,,	7	10	14·6	12	17
,,	26	20	14·7	11	18
		300	14·12	9	21

TABLE 13.—Distribution in respect of length of *T. pecorum* (*T. congolense*).

	Length in microns												
	9	10	11	12	13	14	15	16	17	18	19	20	21
Actual number of each length counted	1	6	17	32	67	52	52	37	25	7	2	1	1
Percentage, to the nearest whole number	...	2	6	11	22	17	17	12	8	2	1	...	...

Measurements were made of the trypanosomes in the blood of three out of the four animals infected with this strain (see Tables 12 and 13). In the original guinea-pig, No. 14, twenty-five trypanosomes were measured on the 1st, 6th, 8th, and 9th days of the infection. The average length of these hundred individuals was 13·37μ; the longest measuring 18μ, and the shortest 9μ. In the ram, and guinea-pig No. 29, it was impossible to find more than one or two parasites in the blood films taken on each of the earlier days of the infection, and so detailed measurements at this stage were out of the question. Twenty trypanosomes were, however, measured, in the case of the ram, on the 16th, 18th, 20th, and 21st days. The average length of these eighty individuals was 14·57μ; the longest measuring 18μ, and the shortest 10μ. In guinea-pig No. 29 twenty trypanosomes were measured on the 18th, 19th, and 20th days of the infection. The average length was 13·96μ, the maximum 17μ, and the minimum 10μ.

EXPERIMENT 5

Twenty-two starved *G. tachinoides* were fed in batches in this experiment on white rat No. 7 during a period of nine days. On the 15th day, that is the 7th day after the last day on which the flies were fed, trypanosomes appeared in the blood of the rat. The infection was a very slight one, and although trypanosomes were usually present in the blood, their numbers were always so small that it was impossible to find sufficient to make a satisfactory study of their measurements. On the 1st, 5th, 6th, and 7th days of the infection ten individuals were found with difficulty, and were measured (Table 12). The average length of these forty trypanosomes was 15·15μ, and the range was from 11μ to 21μ. An additional twenty were measured on the 26th day, and gave an average length of 14·7 μ, the longest measuring 18 μ, and the shortest 11 μ.

One rat, two guinea-pigs, and two dogs were subinoculated from this animal, but did not become infected. The rat itself was alive and apparently well on the 107th day when the experiment was ended. Latterly the trypanosomes had but seldom been found in the blood, and the animal appeared to be recovering from the infection. Phagocytosis of the red cells was observed in this rat.

Although this trypanosome (Pl. XXIII, figs. 24-31) differed somewhat in its morphology and animal reactions from the preceding one, isolated in Experiment 4 (Pl. XXIII, figs. 15-23), it has been included with it here, as there did not appear to be sufficient reason for differentiating it. The parasites were always very scanty, and the smaller forms, which in the other strain became more numerous as the infection increased, and which may be of pathological importance, were rarely seen or entirely absent. The difference between the average lengths of this trypanosome (15·00 μ) and that of the ram (14·57 μ) was, moreover, less than the difference between the strain isolated in Experiment 4 as it appeared in the original guinea-pig (13·37μ), and in the ram (14·57 μ) inoculated from it.

The trypanosomes isolated in these two experiments were of the *T. pecorum* or *T. congolense* type. They were short organisms without a free flagellum, although some forms had the anterior end drawn out to such a fine point as to simulate a free portion (Pl. XXIII, figs. 26 and 27). Morphologically they were identical also with *T. nanum*, a species which was supposed to differ from *T. pecorum* only in being uninfective to the smaller laboratory animals. Bruce (1913) has proved, however, that *T. pecorum* and *T. nanum* are really identical; and Blacklock and Yorke (1913) have recently brought forward evidence to show the identity of *T. congolense* and *T. nanum*. It seems probable that *T. pecorum*, *T. nanum*, and *T. congolense* are really all varieties of the same species.

T. vivax (Pl. XXIII, figs. 11 to 14).

Thirty-six wild *G. tachinoides* were fed daily on a young male goat from December 23rd, 1913, to January 5th, 1914. The goat had been under observation for several weeks, and its blood on repeated examination had always proved negative. The tsetse flies had all been starved for two days before being fed on the goat.

On January 5th, that is on the 14th day, a very few trypanosomes were found in the blood. On the following day the parasites were fairly numerous, and continued to be so until the 19th day. From the 20th to the 25th day they were very rare, but increased slightly in numbers from that date until the 31st day.

Coincident with the appearance of the trypanosomes the goat began to show symptoms of illness. He became unusually quiet, showed a tendency to lie down and sleep, and had a slight nasal discharge. The goat was still alive on the 81st day, and appeared to be in excellent health although still infected with the trypanosomes.

The trypanosomes showed all the morphological features characteristic of *T. vivax*. Twenty trypanosomes were measured on each day from the 2nd to the 6th day of the infection. On the first day, and for several days after the sixth day, it was impossible to find a sufficient number of parasites to measure. The average length of these 100 trypanosomes was 23·74 μ, the longest being 28 μ, and the shortest 19 μ (see Table 14). The percentage distribution in length is shown in Table 15.

Owing to the scarcity of experimental animals it was only possible to inoculate one white rat and one guinea-pig with this trypanosome. Neither became infected. From the morphology of the parasite, and these two experiments, there can be no doubt as to the identity of this trypanosome.

TABLE 14.—Measurements of *T. vivax*.

Day of the infection	Number measured	Length in Microns		
		Average	Minimum	Maximum
2	20	24·3	21	28
3	20	23·6	21	28
4	20	22·95	20	25
5	20	24·45	22	27
6	20	23·4	19	26
	100	23·74	19	28

TABLE 15.— Distribution, by percentages, in respect of length of *T. vivax*.

Length in microns									
19	20	21	22	23	24	25	26	27	28
1	4	4	13	19	23	27	4	3	2

SUMMARY

T. pecaudi (*T. ugandae*), *T. pecorum* (*T. congolense*), and *T. vivax* are transmitted by *Glossina tachinoides* in the Eket district of Nigeria, and were isolated by feeding flies caught in the neighbourhood of Ikotobo on healthy animals.

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EXPLANATION OF PLATES.

PLATE XIX

Fig. 1. Ikot Offiong, a typical village of the Eket district.

Fig. 2. The Obium creek from the Qua Iboe river.

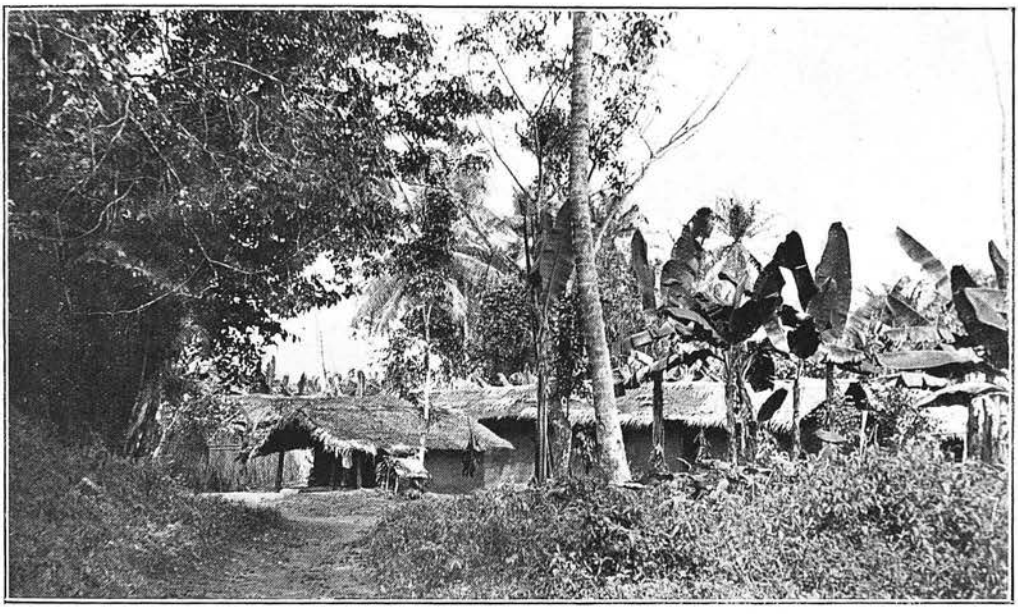

FIG. 1

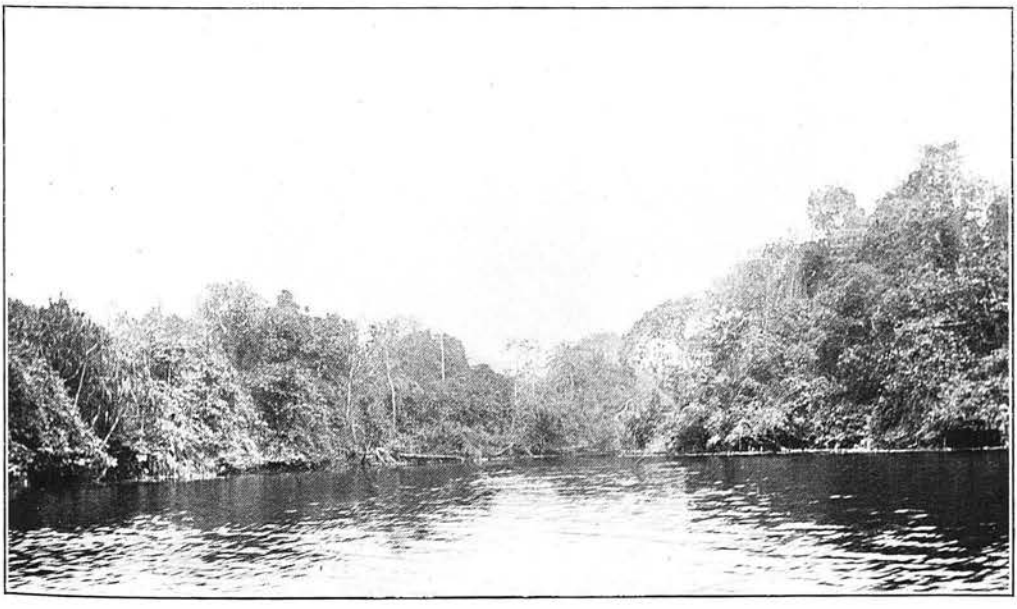

FIG. 2

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PLATE XX

Fig. 3. The dispensary and hospital at the sleeping sickness camp, Ikotobo.

Fig. 4. The instruments used by the native 'doctors' for the excision of lymphatic glands.

Fig. 5. A sleeping sickness patient showing the scars left on the neck by the operation of excision of the glands as practised by the native 'doctors.'

Fig. 6. A few of the huts for the patients of the sleeping sickness camp, Ikotobo.

FIG. 3

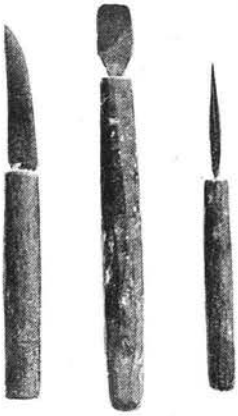

FIG. 4

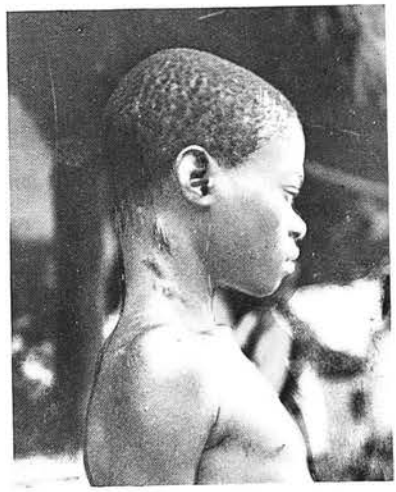

FIG. 5

FIG. 6

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PLATE XXI

Fig. 7. The oldest inmate of the camp.

Fig. 8. A little water-carrier, showing the absence of all protection against the attacks of biting insects.

Fig. 9. A case of sleeping sickness in the somnolent stage.

Fig. 10. A case showing the impetigo of the scalp.

FIG. 7

FIG. 8

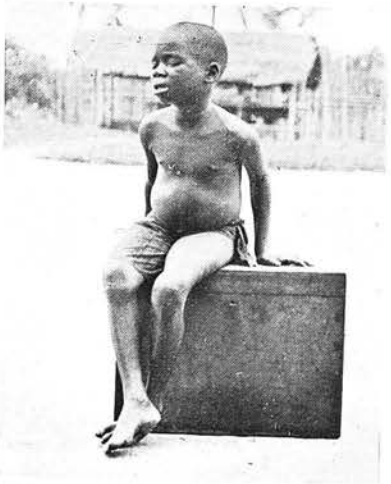

FIG. 9

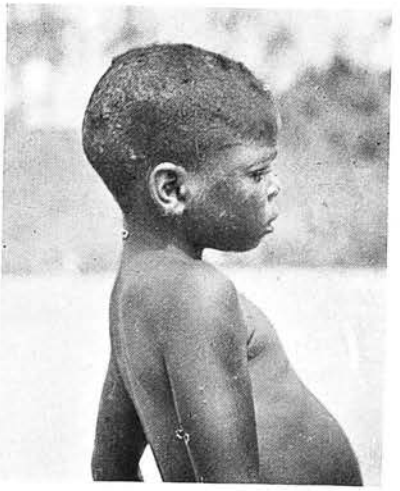

FIG. 10

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PLATE XXII

Cases of sleeping sickness at Ikotobo showing:

Fig. 11. Facial oedema.

Fig. 12. Enlargement of the posterior cervical glands.

Fig. 13. Enlargement of the glands in the parotid regions giving the patient a mumps-like appearance.

Fig. 14. Slight prominence of the eyeballs.

Fig. 15. Oedema of the eyelids.

Fig. 16. The very healthy and happy appearance of many of the patients.

FIG. 11

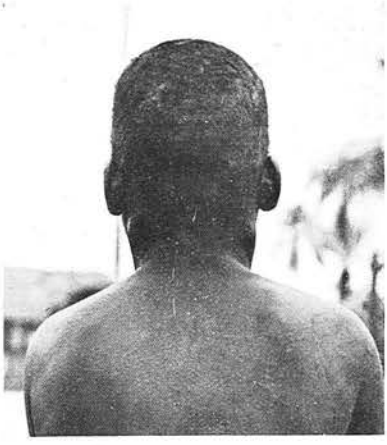

FIG. 12

FIG. 13

FIG. 14

FIG. 15

FIG. 16

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PLATE XXIII

Trypanosomes isolated from wild *Glossina tachinoides*. × 2000.

Figs. 1-6. *T. pecaudi* (*T. brucei* of Uganda) from guinea-pig No. 5. Figs. 4, 5, and 6 illustrate the atypical stumpy forms found in this animal.

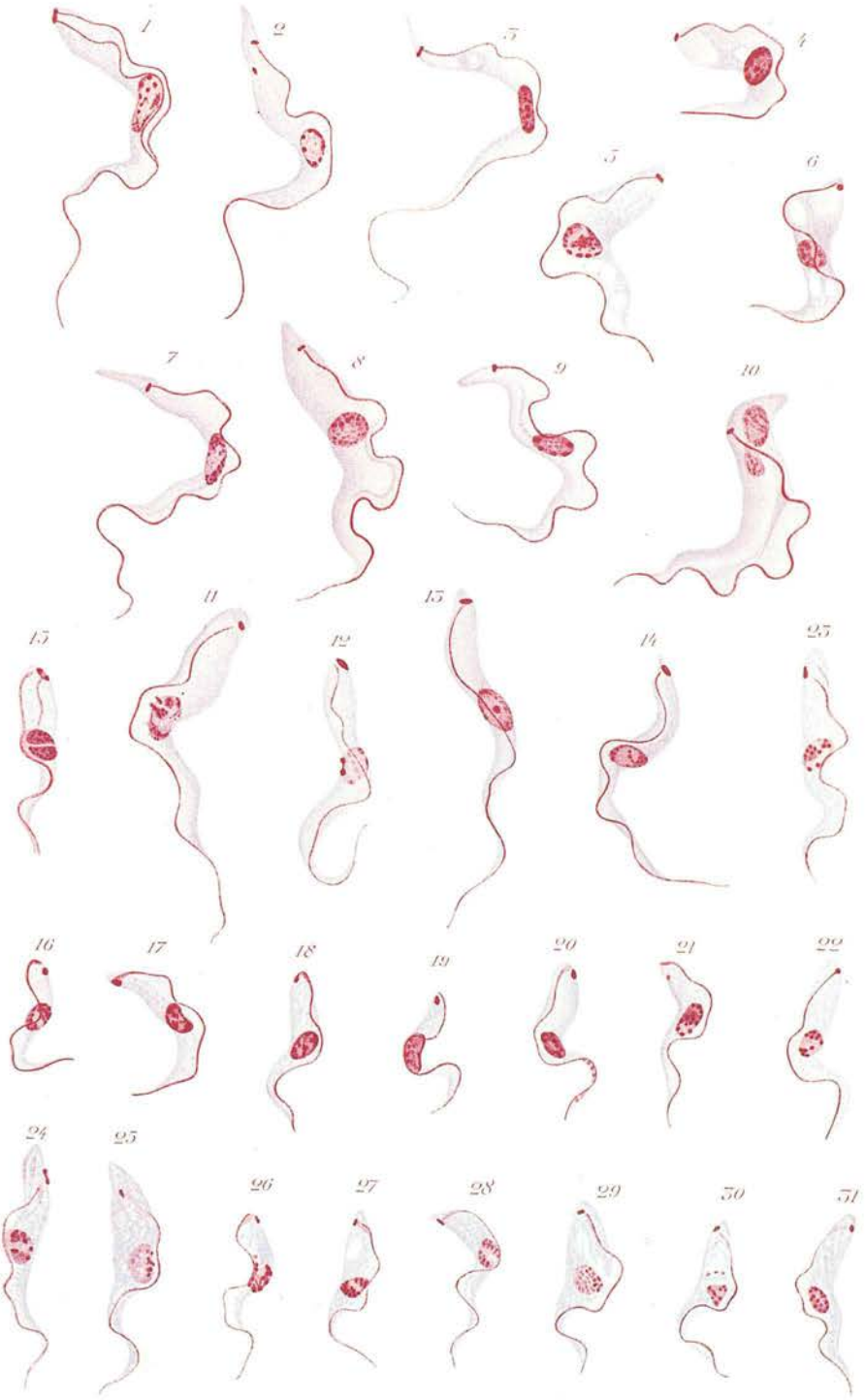
Figs. 7-10. *T. pecaudi* (*T. brucei* of Uganda) from rat No. 16. The same strain of trypanosome as figs. 1-6.

Figs. 11-14. *T. vivax* from goat No. 21.

Figs. 15-21. *T. pecorum* (*T. congolense*) from guinea-pig No. 14.

Figs. 22 and 23. *T. pecorum* (*T. congolense*) from ram No. 28. The same strain of trypanosome as figs. 15-21.

Figs. 24-31. *T. pecorum* (*T. congolense*) from rat No. 7.

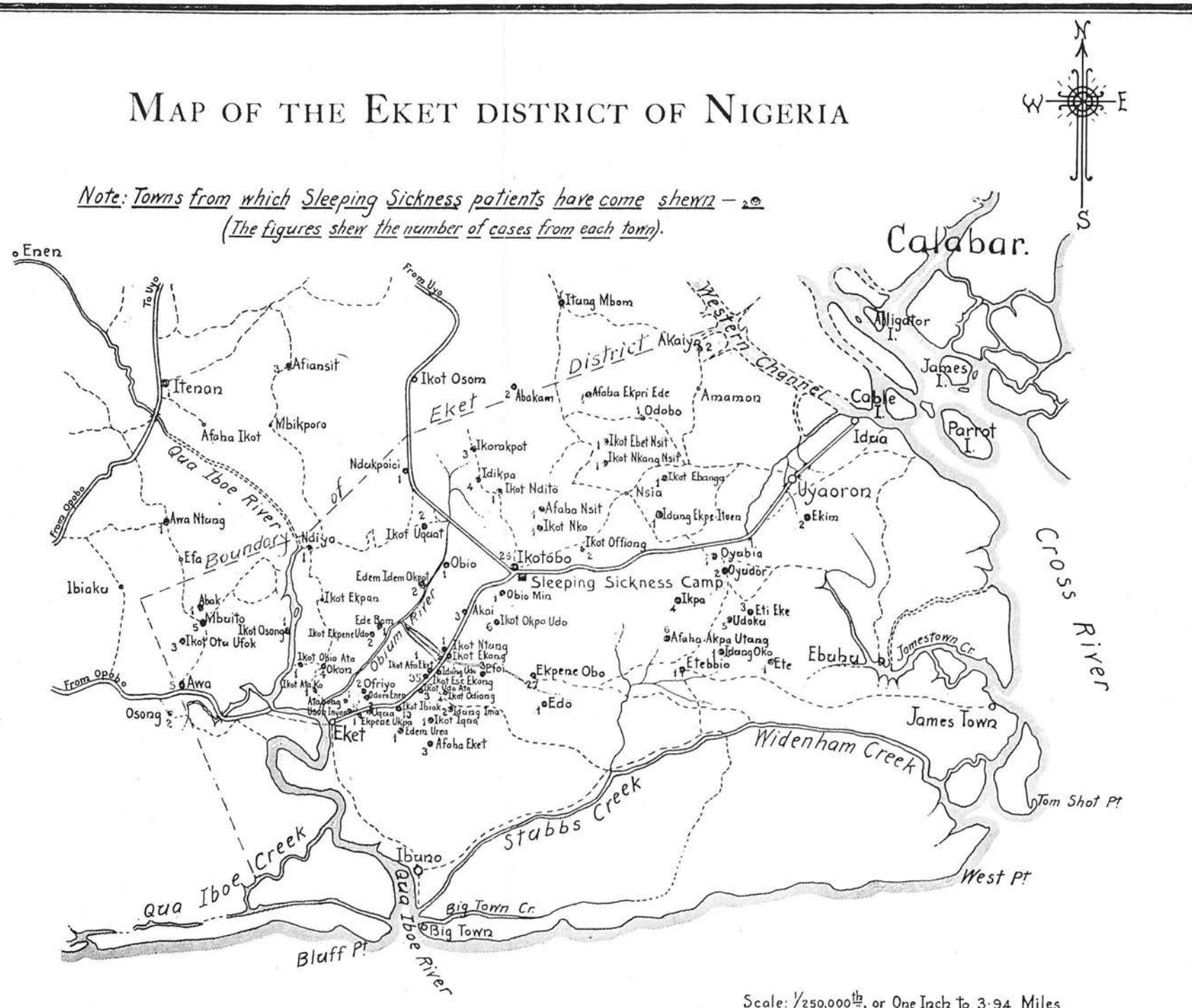


M. Rhodes pinx.

TRYPANOSOME STRAINS ISOLATED FROM

WILD GLOSSINA TACHINOIDES

Map of the Eket district of Nigeria



Prepared from Government Map, and Map of W.C.W. Eakin Esq., F.R.G.S.

January, 1914.

A.V.W. 20-1-14.

Auto-Erythrophagocytosis as an aid to the diagnosis of Trypanosomiasis.

[*Reprinted from* The Journal of the London School of Tropical Medicine, Vol. II., Part III.]

Auto-Erythrophagocytosis as an Aid to the Diagnosis of Trypanosomiasis.

By J. W. SCOTT MACFIE, M.A. (Cantab.), B.Sc. (Edin.),
West African Medical Staff,

and

J. E. L. JOHNSTON, M.B., B.S. (Lond.), D.T.M. & H. (Cantab.),
West African Medical Staff.

(*Preliminary Communication.*)

Auto-erythrophagocytosis has been observed by Connal (1) in the blood in cases of trypanosomiasis, malaria, smallpox, filariasis, and ankylostomiasis, but in the last three it is possible, if not probable, that it was due to an added malaria.

Wright (2) has seen it in two cases of pneumococcal infection, and it has been seen *in vitro* by several observers—Barratt (3), Hektoen (4), and others.

Connal found erythrophages in the blood at the same time as, and in some cases after the disappearance of, trypanosomes, and he mentions one case that showed a single macrophage a month after inoculation, before the appearance of trypanosomes, but he does not state whether the cell contained erythrocytes, nor whether the animal—a guinea-pig—finally developed trypanosomiasis.

We have examined several animals infected with trypanosomes, and in some have noticed the ingestion of red cells. The erythrophages observed resemble those described by Connal. The number of red cells ingested by a single leucocyte varied from one to twelve or even more. Many phagocytes showed red cells partially absorbed, the depth of staining varying inversely with the state of absorption ; some showed apparent vacuoles which we consider may be the final stage ; many showed a large number of granules

evidently derived from digested red cells—perhaps one possible source of the pigmented mononuclears so frequently seen in malaria. The shape of the erythrophage when containing cells is largely determined by the number of its red cells, one or two causing but slight alteration, ten or twelve causing enormous distention and a bloated appearance of the leucocyte. In a few instances a red cell appeared to be placed on the top of the centre of the nucleus.

The ratio of cells containing ingested reds to other leucocytes is, as a rule, very low, seldom over one per cent., but one case (Mouse 31) showed no less than 11·5 per cent. The variation in the percentage ratio of trypanosomes to leucocytes (which we will call for convenience the trypanosome index), whether erythrophages are present or not, appears to be inconstant, but we have several times noticed a drop in the index on the same day as erythrophagocytosis. For example, in Rat 41, from July 20th to July 26th, the index remained persistently high—58 to 119—except on July 25th, when it was 10·5, and in Rat 47, from July 24th to July 28th, the index varied from 86·5 to 173, except on July 25th, when it was 18·5. In each case the low index occurred on the only day on which erythrophagocytosis was observed.

The Infecting Trypanosome.

(1) *T. lewisi.* Connal failed to obtain ingestion of red cells in rats infected with *T. lewisi*, which he attributes to its non-pathogenicity ; we, however, have obtained marked phagocytosis of reds in certain rats that were undergoing treatment, probably owing to a relative rise in the pathogenicity of the trypanosomes.

The other trypanosomes we employed were (2) a trypanosome closely resembling *T. gambiense*, obtained from a case of sleeping sickness.

(3) A trypanosome resembling *T. duttoni*, from a mouse—naturally infected.

We have divided our cases into three groups :—

Group A. In which Leucocytes containing red cells were present in blood which later showed trypanosomes.

1. Monkey 37. Injected with Trypanosome (2).
   Fifth day; showed erythrophagocytosis.
   Seventh day; showed trypanosomes.
2. Mouse 31. Injected with Trypanosome (2).
   Eighth day; intense ingestion of reds.
   Ninth day; trypanosomes appeared, and increased in number whilst the ingestion decreased, until the thirteenth day, when the mouse was accidentally killed.
3. Goat 11. Injected with Trypanosome (2) on June 4th, showed ingestion of red blood cells on July 14th, but it was not until August 5th, twenty-two days later, that trypanosomes were sufficiently numerous to be detected in the peripheral blood.

Group B. In which both were at some time present simultaneously.

1. Rat 40. Injected with Trypanosome (3).
   Third day; a few trypanosomes.
   Fifth day; a few trypanosomes and a few ingested red cells.
   Sixth to twelfth days; no further trypanosomes, but a few scanty ingested reds.

2. Rat 41. Caught infected with *T. lewisi.*
   Ten days later scanty erythrophagocytosis was seen (Index 10·5).
3. Rat 43. Caught infected with *T. lewisi.*
   Second day; phagocytosis very marked.
   Fourth day; unfortunately died.
4. Rat 47. Caught infected with *T. lewisi.*
   Third day; ingestion of several reds (Index 18·5).
   Succeeding days; no further ingestion, though trypanosomes continued
5. Mouse 30. Caught infected with trypanosome (3).
   Second day; one red seen ingested; a few trypanosomes.
   Fourth day; more reds phagocytosed; many trypanosomes seen.
   No further reds were seen phagocytosed; the mouse died on the tenth day.
6. Monkey 21. Inoculated with Trypanosome (2).
   Tenth day; Trypanosomes appeared.
   Eleventh day; Erythrophagocytosis also seen.

Group C. In which phagocytosed red cells were seen after the disappearance of trypanosomes.

1. Rat 40. Also falls under group B.

Relation to Protozoal Diseases.

We have also observed ingestion of red cells in one case of Blackwater fever. A guinea-pig injected with blood from a case of Yellow fever showed a rise of temperature on the second day, accompanied by erythrophagocytosis ; the blood did not contain any parasites of the other diseases mentioned.

We have, however, so far failed to find this in blood from Yellow fever patients. Assuming that the causal organisms of these diseases are of a protozoal nature, as seems probable, there would appear to be a certain relationship between erythrophagocytosis and protozoal diseases such as those mentioned: Trypanosomiasis, Malaria, Blackwater fever, and Yellow fever.

Question of Immunity.

Connal suggests that the phenomenon is related to the development of immunity, but its occasionally initial appearance would seem to negative this theory, unless perhaps it were connected with an abortive attempt at immunisation.

Diagnostic Significance.

We put forward the suggestion that erythrophagocytosis may be of material aid in the diagnosis of trypanosomiasis.

In group A, which may be considered to correspond to early cases, the appearance of trypanosomes in the blood was definitely preceded by phagocytosis of red cells; in group C, or cases of a late stage, phagocytosis was seen after trypanosomes had disappeared.

Moreover, trypanosomes are not by any means invariably to be found in the blood in cases of trypanosomiasis. Trypanosome (2) was obtained from one of several cases met with in Nigeria, in which repeated blood-examinations were negative, though trypanosomes could be readily obtained from

gland-juice or from cerebro-spinal fluid—methods, however, not always practicable with native patients. In such cases, it seems to us, the finding of erythrophagocytosis would be of considerable importance.

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(2) Br. Med. Journ. 1906, vol. i. p. 143.
(3) Proc. Roy. Soc. Lond. 1905, Series B, vol. lxxvi. p. 524.
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Observations on the Action on Trypanosomes of certain Drugs and of Staphylococcus Pyogenes.

[*Reprinted from* THE JOURNAL OF THE LONDON SCHOOL OF TROPICAL MEDICINE, Vol. II., Part III.]

OBSERVATIONS ON THE ACTION ON TRYPANOSOMES OF CERTAIN DRUGS AND OF *STAPHYLOCOCCUS PYOGENES*.

BY J. E. L. JOHNSTON, M.B., B.S. (Lond.), D.T.M. & H. (Cantab.),

AND

J. W. SCOTT MACFIE, M.A. (Cantab.), M.B. (Edin.).

DURING the past year experiments have been carried out to show the toxic effects, if any, of certain drugs and of *Staphylococcus Pyogenes* on Trypanosomes, both *in vitro* and *in vivo*. Connor (1) reports three cases of plague that he treated with intravenous injections of tincture of iodine in dilute solution, all of which recovered. As he points out, the small number of cases obviates the drawing of any possible conclusions, but the results may at least be considered as inciting further investigation.

Davaine found that iodine destroyed the infectivity of septicæmic blood in a dilution of 1 in 10,000.

It seemed, therefore, a matter of interest to investigate the effects of iodine, not on a bacterial infection such as plague, but on a protozoal disease such as trypanosomiasis.

Bromine being of close chemical affinity to iodine was also chosen for experiment, as was osmic acid, from its rapid action in fixing protozoa in blood films.

Experiments in vitro.

A few initial experiments were made to determine the effects of dilute solutions of iodine, bromine, and osmic acid on trypanosomes in cover-slip preparations. For this purpose 1 in 40 solutions of the substances were prepared, and five minims of each were added to ten ounces of water.

A drop of each of these diluted solutions was then added to a drop of guinea-pig's blood containing trypanosomes on three cover-slips, and the effects watched under the microscope. The species of trypanosome employed was that for which one of us has recently suggested the name *T. nigeriense*. In each experiment a control preparation with saline solution was employed.

Only a few experiments were carried out, and the results were somewhat inconclusive, so that it will not be necessary to recount them in detail. As compared with the control, the immediate effect of iodine solution appeared to be a partial laking of the blood, and the trypanosomes tended to assume those atypical forms that have been described as "latent." After thirty minutes but few actively motile parasites remained, and an hour later nearly all the trypanosomes appeared to be dead. The action of the bromine solution was similar, but in some of the experiments it seemed to be transitory. After the first few minutes a good many of the trypanosomes had assumed a tadpole-like shape, but even two hours later there were still a number of actively motile parasites present. The action of the osmic acid solution was perhaps a little more intense, very few living trypanosomes being found in the preparations one hour after the beginning of the experiments.

Experiments in vivo.

The following solutions of the above drugs were made :—

Iodine	Tinct Iodi	m. vii.
	Aq. distil. ad . . .	ʒ i.
Bromine . . .	Brom. (2·5 p. c.) .	m. vii.
	Aq. distil. ad . . .	ʒ i.
Osmic Acid . .	Ac. Osmic (2 p. c.) .	m. xii.
	Aq. distil. ad . . .	ʒ i.

The trypanosomes employed were *T. vivax*, in a sheep, and *T. lewisi*, in rats. As intravenous injections in rats, frequently repeated, are all but impossible, it was decided to administer the drugs to them subcutaneously.

As a slight indication of the number of trypanosomes in the blood, it was decided to take the number observed relative to 100 leucocytes (in every case at least 200 leucocytes were counted), this percentage-ratio being called the "trypanosome index." This index was recognised to be quite inconstant, even under normal conditions, but seemed to be of considerable value as an indication when, if at all, a precise count by the Thoma-Zeiss method should be made, it being difficult to obtain a ready flow of blood daily, for a long period, from a small animal such as the rat.

Experiments with Iodine.

Exp. 1. Rat 41.

Index was 30·76 on the first day, when 20 minims of the solution described above were injected. As this dose appeared to have a marked effect on the rat, the future doses were halved, and 10 minims were injected daily until the 16th day inclusive.

The index remained high but very irregular, varying from 40 on the 13th day to 119 on the 9th ; but on the 11th day it was as low as 10·5. On

this day only, as noted in a previous paper, marked auto-erythro-phagocytosis was observed.

On the 16th day, a slight skin slough commencing, and no effect on the trypanosomes being apparent, the experiment was stopped.

Exp. 2. Sheep 65.

One drachm of pure tincture of iodine was introduced into the external jugular vein of a sheep infected with *T. vivax*. On the 2nd day the sheep was obviously ill, trypanosomes remaining very numerous. Death occurred on the 5th day.

Post-mortem examination showed acute bryncho-pneumonia, with blood-stained pleuræ, a large mass of gelatinous lymph within the pericardium, over the apex of the heart, and intense engorgement of the kidneys.

Boehm and Berg have shown that large doses of iodine will produce œdema of the lung, hæmorrhagic exudation into the pleural cavity, and great renal congestion.

Experiment with Bromine.

Exp. 3. Rat 42.

First day, index 36. As in the first experiment, the initial dose of 20 minims being apparently too large, was followed by daily injections of 10 minims. In contrast with Rat 41, the index remained stationary during the injections, the greatest variation being from 22·5 on the 3rd day to 37·5 on the 6th.

The local effect, however, was more marked, a skin slough commencing the 7th day, whereupon the injections were stopped. The blood was examined daily until the 16th day, but nothing of interest was observed, except some leucocytosis very possibly due to the septic state of the skin.

Experiments with Osmic Acid.

Exp. 4. Rat 43.

Index on the first day was 147, when 10 minims of the solution were injected. On the 2nd day intense leucocytosis was present, with great destruction of leucocytes. Ten minims were again injected. Four hours later the index was 88·23, and there was marked phagocytosis of trypanosomes and red cells, to which further reference will be made. On the 3rd day 5 minims were injected. The blood was thick and tarry, leucocytes less disintegrated, phagocytosis of red cells still marked, but of trypanosomes less so. On the 4th day the rat was obviously dying. There was leucopenia ; trypanosomes were extremely numerous.

After death, a septic peritonitis was found to be present, some of the osmic acid having apparently entered the peritoneal cavity. The subcutaneous tissues at the points of inoculation were blackened and necrosed.

Exp. 5. Rat 47.

To lessen the local action of osmic acid the dose was halved, 5 minims of the solution being given daily, diluted with an equal volume of normal saline. The index, which was 32 on the 1st day, was exceedingly variable, but was not again as low as 32 except on the 3rd day, when it fell to 18·5. On this day only, as on the corresponding day of Rat 41, auto-erythro-phagocytosis was observed.

There was no other noticeable feature, except that on the 8th day there was some leucocytosis and considerable destruction of leucocytes.

The observations were stopped on the 16th day.

The Mechanism of the Destruction of Trypanosomes in the Body.

Opinion is divided as to the mechanism by which trypanosomes are disposed of in the body tissues. By adding trypanosomes to a hanging drop of eel's serum, Laveran and Pettit (2) obtained rapid trypanolysis by the serum. Massaglia (3) injected trypanosomes from guinea-pigs, mice, and rats into the peritoneal cavity of cold-blooded vertebrates. Examination of the peritoneal fluid, lymph, and blood showed trypanolysis, but in no case was phagocytosis observed. Delanoë (4) has described the phagocytosis of the Leptomonas of Kala-azar when injected into the peritoneal cavity of a mouse, and he has also seen phagocytosis of *T. lewisi*. Roudsky (5) has observed the phagocytosis of *T. lewisi* in the peritoneum after injection of immune mice, the trypanosome sticking to the leucocyte by its posterior end, round which the leucocyte sends a sleeve-like prolongation.

Delanoë (6) describes phagocytosis in detail. The trypanosomes are destroyed in full vitality, and if the flagellum happens to be the last portion ingested it may be seen to be motile until the moment of its disappearance.

In our own case the ingestion of trypanosomes was watched in the living blood. In one instance three trypanosomes were observed by the side of a phagocyte, and in spite of violent contortions they appeared unable to get away, although other trypanosomes were seen passing freely in the immediate neighbourhood. Gradually each was destroyed, and all were absorbed by the phagocytes within thirty minutes. In stained films we observed appearances similar to those figured by Brumpt in his 'Précis de Parasitologie,' 1910, p. 124.

The Toxic Effects of certain Bacteria on Trypanosomes.

Levaditi and Twort (7) investigated the action on trypanosomes *in vitro* of *B. coli*, *B. prodigiosus*, *B. mesentericus*, *B. pyocyaneus*, and *B. subtilis*. The first four had little or no effect ; but when 15 drops of a *subtilis* culture were mixed with 2 drops of infected blood at 37° C., trypanolysis was complete in 25 minutes. This effect was proved to be due to a toxin, the filtrates of washed cultures being active, the washed bacilli themselves inactive. They found that the trypanotoxin was not effective in the living blood, possibly because it was seized first by the living cells of the blood.

Rodet, Mdlle. Rubenstein et Bader (8) investigated the effects produced by bacterial infections on the course of an infection with trypanosomes. The trypanosome used was *T. brucei*, and the bacteria were Eberth's bacillus, *B. anthracis*, *Staphylococcus*, and *Streptococcus*.

They found that animals infected with Nagana were more, rather than less, resistant to bacterial infections ; in no case was a bacterial infection of curative value.

Thomas and Breinl (9) stated that guinea-pigs suffering from tuberculosis were more susceptible than healthy guinea-pigs to infection with trypanosomes, but gave no account of their experiments. Orsi (10), on the other hand, proved that the tubercle bacillus had a definitely antagonistic effect on the trypanosome of Nagana, a previous infection with tuberculosis com-

pletely preventing the appearance of the trypanosome in inoculated guinea-pigs. The effect was apparently due to the vital action of the bacillus, tuberculin, killed tubercle bacilli, and ethereal extracts all proving inactive.

Experiments with Staphylococcic Vaccine.

Whilst observations were being made on a rat normally infected with *T. lewisi* it was noticed that the rat showed staphylococci in its blood, and that the trypanosomes rapidly decreased in number and finally vanished, and that this was then followed by recovery of the rat from its staphylococcal infection.

To investigate whether this were any relation of cause and effect, blood was procured aseptically from the rat, cultures of staphylococci grown, and a vaccine was prepared.

Exp. 6. Rat 52.

First day, index 9. Injection of two million staphylococci. This was followed by leucocytosis and a great fall in the trypanosomes, so that an index was barely obtainable. On the 6th day, two millions injected ; there was a temporary rise in number of both leucocytes and trypanosomes on the 7th and 8th day.

On 14th day three and a half millions were injected ; there was a marked increase in the trypanosomes. Further injections of four millions each were given on the 20th, 26th, and 33rd days. The trypanosomes decreased, the index after the 26th day, when it was 4, never being above 1. On the 42nd day no trypanosomes could be found ; but one was seen on the 44th day, and on the 47th they were fairly numerous again.

Exp. 7. Rat 61.

First day, index 115·5 Three million staphylococci were injected on the 1st and 8th days, and four millions on the 11th, 17th, 23rd, and 30th days. There was no obvious effect. There was slight leucopenia, instead of the leucocytosis seen in Rat 52 ; the trypanosome index remained at a high level.

Exp. 8. Rat 62.

First day, index 82·5. Injection of four million staphylococci, which was repeated on the 8th day. Trypanosomes remained very numerous, and the rat died on the 9th day.

Conclusions.

Our results have been almost entirely of a negative character. Of the drugs experimented with, iodine and osmic acid appear to have a very disturbing effect, not only on the trypanosomes, but also unfortunately on the host.

We consider that there can be no doubt that trypanophagocytosis does occur, and that large numbers of trypanosomes may by this means be rapidly destroyed.

Experiment 6 might be construed as meaning that the trypanosomes were reduced by the effects of the staphylococcic vaccine to a vanishing point, and that, on stopping the vaccine before cure was complete, the trypanosomes returned.

However, the vaccine produced no similar effects in Experiments 7 and 8, and it is probable that the variation in the number of trypanosomes seen in Experiment 6 was merely the normal variation of *T. lewisi* over a prolonged period.

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A CASE OF EQUINE TRYPANOSOMIASIS CHARACTERIZED BY THE OCCURRENCE OF POSTERIOR NUCLEAR FORMS.

By J. W. SCOTT MACFIE, M.A., M.B., Ch.B.

West African Medical Staff.

AND

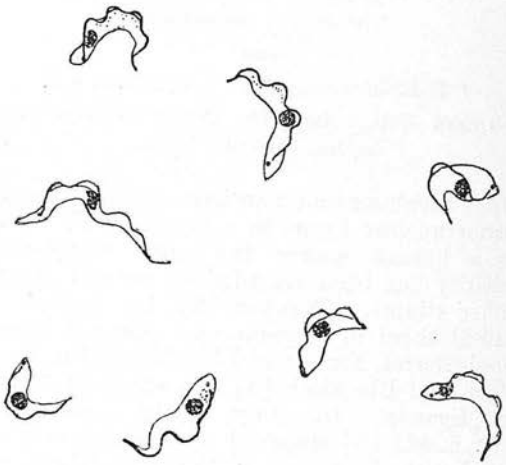
J. E. L. JOHNSTON, M.B., B.S., D.T.M. & H.

West African Medical Staff, The Medical Research Institute, Lagos, Southern Nigeria.

SINCE Stephens and Fantham [1] first described posterior nuclear forms in a trypanosome obtained from a human source the same morphological peculiarity has been recorded by several observers in other strains. Wenyon [2], for example, has described them in *Trypanosoma pecaudi* from the Bahr-el-Ghazal, Yorke and Blacklock [3] in *T. equiperdum*, and Blacklock [4] in a strain of *T. brucei* from Uganda. In July, 1912, one of us (J. W. S. M.) [5] observed these forms in a horse infected with *T. brucei* in Northern Nigeria; but with this exception, so far as we are aware, the occurrence of trypanosomes showing this morphological peculiarity has not been described from West Africa. We think, therefore, that the observations recorded in this note may be of interest.

Last July a series of blood slides was sent to the Medical Research Institute, Lagos, by Dr. Hutton.

from Accra, on the Gold Coast. The blood films had been taken from two horses infected with trypanosomiasis. On examining them we found that the parasites were of the *T. brucei* (*pecaudi*) type, a species of trypanosome that is common throughout West Africa, and which accounts annually for a heavy mortality amongst domestic animals. The one horse had few trypanosomes in the blood, but in the other they were numerous, and on going over



T. brucei (*pecaudi*) from a horse at Accra, Gold Coast. × 1,000.

the slides it was observed that many of them had the nucleus situated in the posterior half of the body.

The accompanying drawings have been made from a film from the latter horse. They were made with a camera lucida, and the magnification was in each

case 1,000 diameters. The usual long and slender forms and short and stumpy forms are illustrated, and in addition sketches are given of several forms in which the nucleus occupied a more or less posterior position. No forms were met with in which the trophonucleus was actually posterior to the kinetonucleus, although it was not infrequently seen almost touching it.

As there appeared to be an unusually large number of forms with the nucleus posterior, a thousand trypanosomes were examined with a view to determining the percentage. In doing this the procedure of Blacklock [6] was carried out. "Every trypanosome met with was counted, whether long, intermediate, or short, dividing or non-dividing. The number of posterior nuclears was noted, non-dividing forms only being chosen." The posterior nuclear forms were also classified, according to his convention, into his forms A, B, and C. The results were as follows:—

Trypanosomes enumerated	Posterior nuclear forms		
	A	B	C
1,000	30	17	1

There were thus 4·8 per cent. of the trypansomes in which the nucleus was definitely posterior. We do not know on what day of the infection the blood films we examined were taken, but such a percentage is certainly a high one. Stephens and Fantham (*loc. cit.*) found that in rats infected with *T. rhodesiense* these forms might form about 6 per

cent., and Wenyon and Hanschell [7] as much as 72 per cent. of the parasites present.

The occurrence of posterior nuclear forms in the various species of trypanosomes mentioned above has been used as an argument against the specificity of *T. rhodesiense* [8], and it has recently been asserted that *T. rhodesiense* is *T. brucei* [9].

As it was not possible to examine the animal reactions of the trypanosome described above we cannot positively identify the species, but it presented the same morphological appearances as the parasite that accounts for a large number of deaths amongst horses every year in Northern Nigeria, several examples of which were identified for one of us by Sir David Bruce as *T. brucei* [10]. As the trypanosome is undoubtedly polymorphic, the species is probably that for which Stephens and Blacklock have proposed the name *T. ugandæ* [11]. This species and *T. pecaudi* are by some authorities considered to be the same.

In Nigeria, and we believe in other West African Colonies, *T. brucei* (*pecaudi*) is a common parasite of domestic animals. If, on account of the fact that posterior nuclear forms are common to both, we are to believe this parasite identical with *T. rhodesiense*, it is a curious fact that human trypanosomiasis in West Africa appears to differ materially from the disease in Rhodesia. If *T. rhodesiense* is *T. brucei* (*pecaudi*), we would expect to find cases of human trypanosomiasis due to *T. rhodesiense* in the places where *T. brucei* was common in the domestic animals. This is not the case. In Nigeria, at any rate, sleeping sickness does not present the acute features it does in Rhodesia. The disease is endemic, but by no means invariably fatal. The trypanosome from a case of sleeping sickness in

Southern Nigeria has recently been studied by one of us. The results have not yet been published, but it may be of interest to state here that neither in its morphology, its measurements, nor its animal reactions does it resemble either *T. brucei* (*pecaudi*) or *T. rhodesiense*.

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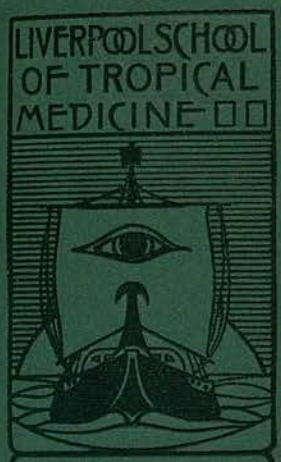

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A Note on the Occurrence of Spirochaetosis of Fowls in Southern Nigeria

J. W. SCOTT MACFIE
AND
J. E. L. JOHNSTON

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A NOTE ON THE OCCURRENCE OF SPIROCHAETOSIS OF FOWLS IN SOUTHERN NIGERIA

BY

J. W. SCOTT MACFIE, M.A. (Cantab.), M.B., Ch.B. (Edin.)
(WEST AFRICAN MEDICAL STAFF)

AND

J. E. L. JOHNSTON, M.B., B.S. (Lond.), D.T.M. & H. (Cantab.)
(WEST AFRICAN MEDICAL STAFF)

PLATES II, III

(*Received for publication* 12 *December*, 1913)

Spirochaetosis of fowls, first described by Marchoux and Salimbeni,[1] in Brazil, and subsequently studied by a number of workers, notably by Balfour,[2] occurs in many countries. In Africa the disease has been recorded in Khartoum by Balfour,[3] in Southern Rhodesia by Bevan,[4] in the neighbourhood of Tunis by Comte and Bouquet,[5] in Senegal and Somaliland by Brumpt,[6] in the French Soudan by Bouet,[7] in the vicinity of Cape Town by Jowett,[8] and in the German Cameroons by Mohn.[9] But so far as we are aware, its occurrence in British West Africa has not hitherto been chronicled.

In August, 1913, it was noticed that a fatal disease was attacking the poultry kept at Iju, near Lagos, in Southern Nigeria. Both ducks and fowls were dying off rapidly, and from the symptoms described, it was suspected that the disease would prove to the spirochaetosis. Thanks to the kindness of Mr. Harris, we were able to examine the bodies of several of the birds that had succumbed, and later on to study the disease in both fowls and Muscovy ducks.

On making enquiries in the neigbourhood, it was found that the disease was well known to the natives. Poultry rearing is almost universal in the villages of Nigeria, and all the natives questioned regarding the disease professed to be well acquainted with it. They recognised that when once it appeared it spread

rapidly amongst their fowls, but they did not associate the disease with any particular condition, unless it were with the cold weather that accompanies the rainy season. Their treatment consisted in rubbing the legs of the infected birds with shea butter, a measure that had proved quite unsuccessful in the cases that came under our notice.

SYMPTOMS

The symptoms observed were those generally described in this disease, and were fever, diarrhoea, anaemia, somnolence, and paresis. The accompanying photographs (Plate II) illustrate the aspect of the infected birds At first they showed only slight symptoms of illness although the blood might be swarming with spirochaetes. The comb appeared to be unusually pale, there was slight diarrhoea, and the birds showed a tendency to stand for long periods with their feathers ruffled and their heads sunk between their shoulders. When they moved, their gait was staggering. Later the anaemia deepened, somnolence became a conspicuous feature, and paralysis of the wings, and especially of the legs, followed. Finally, the birds were unable to stand, and lay prone on the ground until death occurred. The appetite remained good almost up to the end.

The disease first showed itself in some Muscovy ducks, but subsequently spread to the fowls kept in the same compound. Some of the birds attacked were chickens. All the infected fowls were thoroughly searched for ticks and other parasites, but none were found with the exception of a few fleas on the combs which have been identified by Mr. N. C. Rothschild as *Echidnophaga gallinaceus*.

POST-MORTEM APPEARANCES

After death the bodies of the birds were found to be wasted, and the tissues unnaturally pale. The spleen was always considerably enlarged. The most conspicuous feature was, however, the condition of the liver which was greatly enlarged, and in which necrotic patches occurred sometimes of considerable size. In one case a large blood clot was found in the stomach.

MORPHOLOGY OF THE PARASITE

But little need be said about the morphology of the spirochaete, since it appeared to be very similar to that described by Balfour in the case of the Sudanese strain. The general appearance of the parasites is shown on Plate III (Figs. 1, 2). The spirochaetes varied greatly in length, and in the number of their spirals. Some forms were as short as 9μ, and had only three or four spirals, others were as long as 27μ or more, and had twelve or fourteen spirals. The latter were of the combined type, and appeared to consist of two spirochaetes connected by a very fine filament. In some cases the spirals were close, in others they were open. One end of the body was often curled up. The bodies of the spirochaetes were pointed at each end, and in some individuals chromatic dots were clearly visible. In others breaks or gaps occurred in the central core. The breadth of the body varied considerably, some of the parasites being very much stouter than others. Some forms which appeared at first sight to be stout individuals proved on more careful examination to be made up of two spirochaetes. The appearance suggested the result of a recent longitudinal division.

BLOOD CHANGES

In addition to the condition of the blood described above, certain changes in the leucocytes were observed. Large vacuolated mononuclear cells were commonly met with, and ingestion of red blood corpuscles was seen to have occurred. Both these phenomena have been previously observed by Levaditi,[10] and need not be further discussed. Figs. 3 to 6 on Plate III illustrate the condition described.

INOCULATION INTO MAMMALS

Marchoux and Salimbeni found that guinea-pigs were insusceptible to the parasite of Brazilian septicaemia of fowls, and Balfour failed to infect gerbils by inoculations with the Sudan strain. The latter author concludes that 'this is an avian spirochaetosis, non-transmissible to mammals.' But Levaditi and Lange have shown that fowl spirochaetes may produce a benign infection in rabbits, and Deutz[11] has succeeded in infecting mice.

In our experiments one rat was inoculated subcutaneously with blood from a Muscovy duck, in which countless spirochaetes were present. The animal never showed the parasites in its blood, and no signs of spirochaetal infection were observed.

ACTION OF ATOXYL AND SALVARSAN

Both atoxyl and salvarsan have been proved to give satisfactory results in the treatment of spirochaetosis of fowls in other countries. In our investigations we employed the former drug in three cases, and the latter in two.

ATOXYL. Three fowls were treated by subcutaneous injections of 3/50 gr. atoxyl.

Fowl 99. On August 29th, 1913, the first injection was given. At this time the fowl showed definite symptoms of the disease. The comb was very pale, there was diarrhoea and somnolence, and the gait was unsteady. Spirochaetes were numerous in the blood, and some clumps were present. The next day no parasites could be found in the blood, and they did not reappear subsequently. The inoculation was repeated on the 4th and 7th days. By the 11th day the bird seemed to have regained its normal condition, and a month later was still apparently quite healthy.

Fowl 100. A young bird showing well-marked symptoms—anaemia, ruffled feathers, diarrhoea and uncertain gait. Spirochaetes scanty. The first injection was given on August 31st, and was repeated on the 3rd and 6th days. No spirochaetes were seen after the first injection, and the bird rapidly recovered, and appeared to be in normal health by the 10th day.

Fowl 101. A young bird showing well-marked symptoms. Spirochaetes very numerous in the blood. The first injection was given on August 31st, and the dose was repeated on the 3rd and 6th days. Spirochaetes continued to be present in the blood until the 6th day. The day after the second injection the blood was found to contain masses of tangled spirochaetes. No parasites were seen on subsequent days, but the bird continued to show symptoms of the disease, and died on the 11th day after the first injection.

In the first two cases atoxyl appeared to effect the cure of the disease. In the third, either the condition had advanced too far before treatment was begun, or the dose of atoxyl was insufficient to destroy the parasites before they had effected mortal injuries on the host. In spite of the injections the disease progressed to the so-called 'crisis,' the spirochaetes agglutinated into clumps, and vanished from the peripheral blood, and eight days later the bird died.

SALVARSAN. Two fowls were treated with subcutaneous injections of salvarsan triturated with oil. The dose employed was in each case 0·002 gm.

Fowl 97. The fowl showed well-marked symptoms of spirochaetosis, and the blood was swarming with parasites. The first injection was given on August 30th, and was repeated on the 4th and 7th days. A few spirochaetes were found in the blood on the day after the first injection, but none was detected subsequently. During the first few days the paralysis of the legs and the wings progressed until the bird was unable to stand, then some improvement began to take place so that the fowl was able to move about unsteadily by the 11th day. The other symptoms began to improve simultaneously. Six weeks later the bird appeared to be in good

health in every respect excepting that the paresis of the wings and legs persisted. No marked improvement in this latter symptom has taken place during the last month.

Fowl 98. The bird appeared to be in an advanced stage of the disease, it was quite unable to stand, and lay helpless on the ground. The blood contained countless spirochaetes. The first injection of salvarsan was given on August 30th, and the dose was repeated on the 4th and 7th days. Spirochaetes were still numerous the day after the first injection, and a few were present on the third day. From this time onwards no more parasites were seen. The condition of the bird began to show definite improvement from the 6th day, and by the 11th day recovery was well nigh complete. Six weeks later the fowl appeared to be in perfect health.

In both these cases salvarsan appeared to save the life of the bird. In the case of Fowl 98, this result was remarkable considering the stage to which the disease had advanced before treatment was begun, and the failure of atoxyl to prevent death in the case of Fowl 101, which was not in so serious a condition. This observation corresponds with those of Dschunkowsky,[12] who found salvarsan to be more active than atoxyl in the treatment of spirochaetosis of geese. The persistence of paresis after recovery from spirochaetosis was observed as a sequel to the illness by Marchoux and Salimbeni.

THE 'AFTER PHASE' BODIES OF BALFOUR

Balfour has described certain appearances in the red blood corpuscles of fowls suffering from spirochaetosis which he has termed 'after phase bodies,' and which he considers represent a stage in the development of the parasite. They occur especially during the post-critical stage of the disease, at a time when free spirochaetes cannot be detected in the blood by the ordinary methods of examination. They have been described by Jowett in the spirochaetosis in the vicinity of Cape Town, as well as by Balfour in the Sudanese strain.

We have naturally looked for these bodies with considerable care in the cases that we have studied in Nigeria. We have not, however, succeeded in convincing ourselves of their presence. In a few corpuscles we have seen bodies not unlike those figured by Balfour, but they have shown little or no structure, and might be accounted for by some other cause. Even if these bodies were the 'after phase bodies' of Balfour, they occurred in such small numbers as to constitute a marked difference between the Sudanese and Nigeria strains.

Several species of parasites seem to be concerned in the condition known generally as spirochaetosis of fowls. The parasite of the Brazilian septicaemia, named by Stephens and Christophers *Sp. gallinarum* (= *Sp. marchouxi*, Nuttall),[13] appears to be the most common, and is by some authors considered to be the only species. Brumpt, however, has distinguished the Tunisian strain and the Senegal strain from this species on account of immunity reactions, and has named the former *Sp. nicollei*, and the latter *Sp. neveuxi*. Balfour also has reluctantly decided that the Sudan strain constitutes a new species for which he proposes the name *Sp. granulosa*. In addition to these species, the spirochaete found by Sakharoff attacking geese in the Caucasus has been called *Sp. anserina*. This parasite is, however, capable of infecting fowls, producing in them a disease similar to that caused by *Sp. marchouxi*.

We have been unable to investigate the immunity reactions of the spirochaete infecting poultry in Nigeria, so that it is impossible to determine exactly the species to which it belongs. Morphologically, the parasite appears to be identical with *Sp. marchouxi*, and the symptoms of the disease, and the post-mortem appearances are similar to those described in the case of Brazilian septicaemia of fowls. In several respects the disease differs from that described by Balfour in Sudanese fowls.

It would not be unnatural to expect that fowl spirochaetosis in Brazil and in West Africa might be due to the same species of parasite. In the days when slaves were exported from West Africa there must have been constant communication between the two countries, and it is not unlikely that fowls, some of which may have been infected with spirochaetes, were carried over to Brazil. It is interesting to note in this connection that Blaizot,[14] as quoted by Balfour, believes that from a study of the question of immunity, 'it may yet be possible to affirm that Africa is the original country whence avian spirochaetosis spread.'

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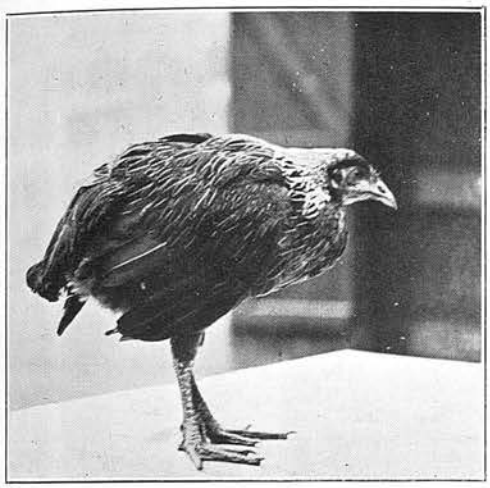
EXPLANATION OF PLATES

Avian spirochaetosis.

PLATE II

Fig. 1, 2, 4, and 5. Fowls in various stages of the disease. Note the attitude, the ruffled feathers, the somnolence, and the paresis of the wings and legs.

Fig. 3. Muscovy duck in a late stage of the disease, and unable to stand on its legs.



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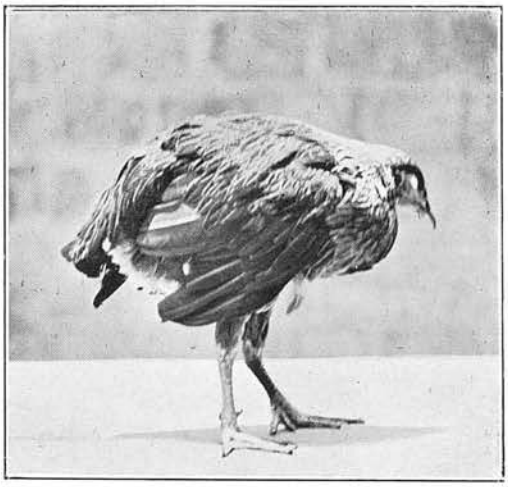

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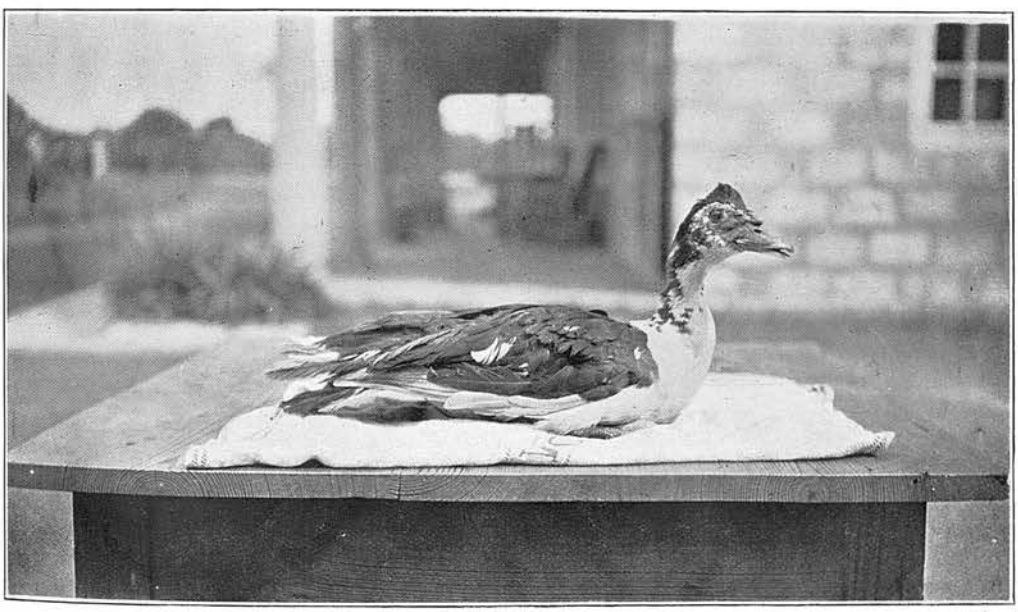

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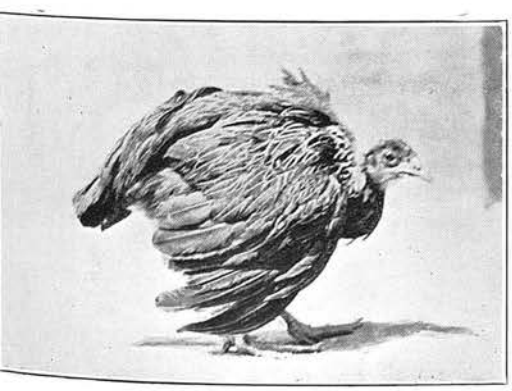

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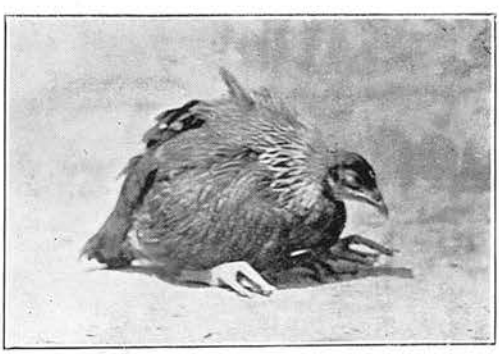

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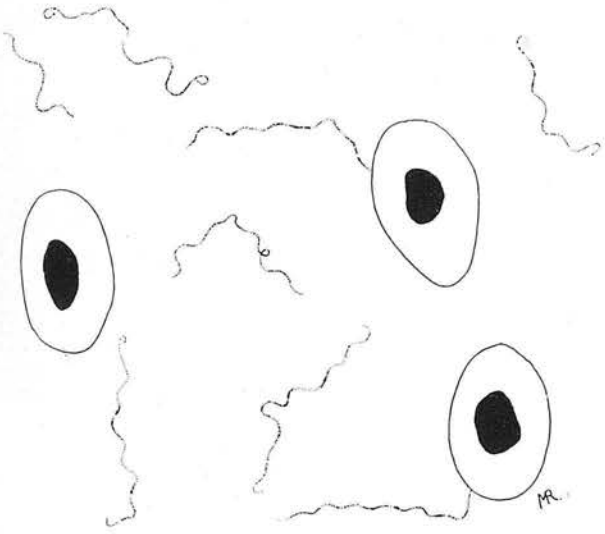
PLATE III

Fig. 1. Spirochaetes, long and short, some with few, some with numerous spirals. Chromatin dots seen in some parasites.

Fig. 2. Group of coiled Spirochaetes.

Figs. 3, 4. Vacuolation of mononuclear leucocytes.

Figs. 5, 6. Auto-erythro-phagocytosis in duck's blood.



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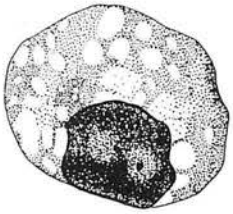

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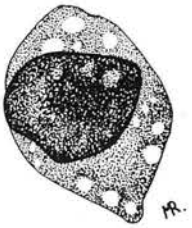

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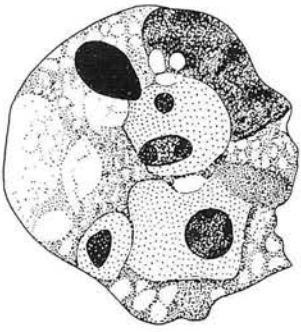

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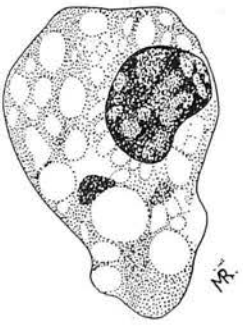

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Experiments and Observations on Yellow Fever

BY

J. W. SCOTT MACFIE

AND

J. E. L. JOHNSTON

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EXPERIMENTS AND OBSERVATIONS ON YELLOW FEVER

By J. W. Scott Macfie, M.A. (Cantab.), M.B., Ch.B. (Edin.), *West African Medical Staff*, and J. E. L. Johnston, M.B., B.S. (Lond.), D.T.M. & H. (Cantab.), *West African Medical Staff*.

The experiments and observations, of which an account follows, were undertaken by us at the Medical Research Institute, Yaba, near Lagos, S. Nigeria, during the course of an epidemic of Yellow Fever at Lagos, which commenced in May, 1913, and is still (September, 1913) in progress.

'It would be strange . . . .' wrote Sir Rubert Boyce (1911), 'if a colony like Southern Nigeria, where the prevailing town mosquito is the *Stegomyia calopus*, and close around which the existence of yellow fever has been officially chronicled during the past fifteen years should be exempt from endemic yellow fever'; and he proceeds to express his opinion that yellow fever is endemic, and that 'the disease has long been overlooked and mistaken for other diseases.' With regard to Lagos itself, Sir Rubert Boyce states that in his opinion undoubted cases of yellow fever occurred in 1894-5, and again in 1902-5. Elsewhere, he says, 'Examination of the medical notes in the hospital at Lagos convinces me that genuine well-marked cases of yellow fever occurred in the years 1902-5; there were also many mild cases,' and 'When it is recollected how little is known of the fevers amongst the 60,000 native inhabitants of Lagos, and when it is understood that by far the most abundant mosquito is the *Stegomyia*, it is not unreasonable to assume that the natives in all probability suffer from a mild type of yellow fever, and that, therefore, yellow fever is endemic.' It is also of interest to note that Syrian residents in Lagos, have long recognised a fatal disease that attacked their countrymen soon after their arrival, a disease that was characterised by remittent fever, vomiting and jaundice.

In May, 1913, a case of undoubted yellow fever occurred in a European at Lagos, and five suspicious cases, four in natives and one in a European, followed at about the same

time. Two months later another series of cases occurred. Two of the patients were Europeans, three Syrians, and twelve natives. During the latter outbreak we had the opportunity of carrying out the experiments which form the subject of this paper.

Observations on Man

Symptoms

A short account should be given of the symptoms presented by the patients suffering from yellow fever at Lagos before proceeding with the experiments that form the particular subject of this article. We are greatly indebted to Dr. Leonard for putting at our disposal the careful records he kept of the cases, from which we have compiled the following summary.

The cases fall chronologically into two groups. The first six occurred in May, and the remaining seventeen in July and August. Each group was ushered in by a fatal case in a European. Taking the cases as a whole up to the date of writing (September, 1913), four have occurred in Europeans, sixteen in natives, and three in Syrians. It is, perhaps, a fact of some significance that six of the native cases occurred in Kroo-boys.

In Europeans the disease presented all the classical features. Two of the cases terminated fatally, and the pathological and post-mortem appearances confirmed the clinical diagnosis. Jaundice was observed on the fourth or fifth day of the disease; vomiting, described as 'black' in two and 'brown' in one, occurred in three out of the four cases, and melaena, and haemorrhages from various mucous membranes, were noticed in the same three patients.

Of the three Syrians attacked, two died on the fifth day of the disease, and the third case, though not fatal, was of a serious type. Vomiting was a prominent symptom in each, and all the typical symptoms were observed in the fatal cases. The organs from the fatal cases were found on examination to present the histological changes that one would expect to find in cases of yellow fever.

TABLE I.—Yellow Fever Cases in Lagos, 1913.

Case			Symptoms and Signs														
No.	Race	Result	Mild or severe	Duration in days	Chills or rigors	Headache	Pains in loins and limbs	Conjunctival injection	Day on which jaundice appeared	Epigastric tenderness	Vomiting	Melaena	Faget's sign or slow pulse	Albuminuria	Number of days of Albuminuria	Haemorrhages from gums
	EUROPEANS—															
14	British	D	S	5	...	+	+	+	4	+	+	+	+	+	...	+
36	German	R	M	9+	...	+	+	+	+...	−	+	+	...	+	...	+
37	British	D	S	5	+	+	+	+	5	+	+	+	+	+	4	+
44	British	R	M	18	+	+	+	+	4	+	−	−	+	+	5	−
	OTHER NON-NATIVES—															
38	Syrian	D	S	5	+	+	+	+	5	+	+	+	+	+	3	+
39	Syrian	R	S	23	+	+	+	+	5	+	+	−	+	+	5	+
41	Syrian	D	S	5	+	+	+	+	4	+	+	+	+	+	(?)4	+
	NATIVES—															
23	Egba	R	M	25	+	+	+	+	9	+	−	−	+	+	...	−
24	Yoruba	R	M	20	+	+	+	+	8	−	−	−	−	+	...	−
25	Ibo	R	M	21	...	+	+	+	8	+	−	−	+	+	...	−
35	Kroo-boy	R	M	22	...	+	+	+	+...	+	−	−	...	+	...	−
40	Yoruba	R	S	26	+	+	+	+	7	+	−	−	+	+	4	−
42	Kroo-boy	R	M	19	+	+	...	+	7	−	−	−	+	+	7	−
43	Yoruba	R	M	24	+	+	+	+	7	+	−	−	+	+	7	−
45	Benin	R	M	17	+	+	+	+	5	−	−	−	+	+	5	−
46	Kroo-boy	R	M	23	+	+	...	+	3	−	−	−	+	+	6	−
47	Yoruba	R	M	12	+	+	...	+	6	−	−	−	+	+	7	−
51	Kroo-boy	R	M	16	+	+	+	+	6	−	−	−	+	+	5	−
52	Kroo-boy	R	M	15	+	+	+	+	5	−	−	−	+	+	5	−
53	Kroo-boy	R	M	13	+	+	+	+	6	+	−	−	+	+	5	−
55	Yoruba	R	M	21	...	+	+	+	8	−	−	−	+	+	4	−
56	Yoruba	R	M	15	+	+	+	+	6	+	+	−	+	+	4	−
63	Sobo	R	S	25	+	+	+	+	11	+	−	−	+	+	6	−

R = recovery ; D = death ; M = mild ; S = severe ; + = present ; − = absent ; ... = no observation.

In the native cases, some of which were complicated by a concurrent malarial infection, the symptoms were variable, and, as a rule, not severe. In many instances the disease was characterised solely by fever, jaundice, and albuminuria. In practically all the native patients the disease was of a mild type. There were no deaths. One of the most severe cases (No. 40) was detected in the routine examination of the passengers travelling on the railway trains. This patient apparently felt quite able to undertake a long journey. The fever was moderate in degree; the premonitory symptoms only such as occur in natives with any form of pyrexia; epigastric tenderness was by no means a constant symptom; vomiting occurred in only one case; and melaena and haemorrhages were never observed. Jaundice was perceptible as a rule on the sixth or seventh day; but this symptom is difficult to observe in a negro, and must be considered somewhat unsatisfactory. The same remarks apply to conjunctival injection. A secondary low pulse rate, or a definite Faget's sign, was almost invariably present. Albuminuria, which persisted for from four to seven days, was the most important symptom, and was present in every case. According to the opinion of those who have had the best opportunity for making the observations, it is rare in Nigeria to find albuminuria in a native suffering from malaria, at any rate, with the degree of fever met with in these cases.

The symptoms in natives were therefore far from typical. This, however, is rather the rule than the exception. Seidelin (1912) writes: 'The occurrence of typical cases in natives is, however, always exceptional. This fact is, according to the now generally accepted opinion, due to the coincidence of two circumstances: that yellow fever in children as a rule is observed in atypical, and often benign forms, and that practically all natives are attacked in childhood.' Seidelin is here referring to his experiences in Yucatan, but it seems probable that his statements would apply equally well to West Africa. It has not been possible hitherto to investigate the disease of children in West Africa

in a satisfactory manner, owing to the reluctance of the natives to consult European medical officers in regard to the so-called 'childish ailments.' It is probable that until certification of the cause of death is made compulsory in the larger towns of Nigeria the matter must remain undecided. Under these circumstances, as Seidelin points out, the demonstration of the supposed parasite would be of the utmost importance in studying the anomalous features of the epidemiology of yellow fever.

THE OCCURENCE OF *Paraplasma*-LIKE BODIES IN THE BLOOD

We have had the opportunity of examining the blood films from nineteen cases of yellow fever (see Table 2); in sixteen we have detected *Paraplasma*-like bodies in the red blood cells. The films were stained deeply, in most cases by Leishman's method, but in a few by Giemsa's. The single blood films forwarded to us from two cases (Nos. 41 and 44) were, unfortunately, unsuitable for examination for minute parasites, and the same remark applies to the films received from case No. 54.

In our earlier examinations we observed some simple ring-shaped bodies that stained blue. These were present, not only in the red blood corpuscles, but also free in the plasma. No chromatin granule could be detected, and although this fact would have been hard to explain were these bodies of a parasitic nature, we considered that the question of their occurrence was worthy of further investigation. Seidelin (1911) has figured bodies presenting very much the same appearance, and has called them 'protoplasma-bodies without chromatin.' He has suggested that 'the absence of a definite chromatin staining . . . . is probably a phenomenon of degeneration.' In one of the first cases examined these bodies were common; but in subsequent cases, although they were occasionally met with, they did not occur with that regularity that one would have expected had they been definitely associated with the cause of the disease. We are therefore inclined to think that they are not of great importance, and although we consider that they may

be associated with yellow fever in some manner that is not understood, we do not believe that they are directly concerned in the aetiology of the disease.

TABLE II.—Synopsis of the Yellow Fever cases examined.

Commission No.	Place	Race	Result	Remarks
37	Lagos.....	British	Death	*Paraplasma*-like bodies present in the blood
38	,,	Syrian	Death	,, ,,
39	,,	Syrian	Recovery ...	,, ,,
40	,,	Yoruba	,,	,, ,,
41	,,	Syrian	Death	Blood films unsuitable for examination for minute bodies
42	,,	Kroo-boy	Recovery ...	*Paraplasma*-like bodies present in the blood
43	,,	Yoruba	,,	,, ,,
44	,,	British	,,	Blood films unsuitable for examination for minute bodies
45	,,	Beni	,,	*Paraplasma*-like bodies present in the blood
46	,,	Kroo-boy	,,	,, ,,
47	,,	Yoruba	,,	,, ,,
51	,,	Kroo-boy	,,	,, ,,
52	,,	Kroo-boy	,,	,, ,,
53	,,	Kroo-boy	,,	,, ,,
54	Aro	Syrian	,,	Blood films unsuitable for examination for minute bodies
55	Lagos.....	Yoruba	,,	*Paraplasma*-like bodies present in the blood
56	,,	Yoruba	,,	,, ,,
62	Forcados	Igabo	,,	,, ,,
63	Lagos.....	Sobo	,,	,, ,,

In all those cases of yellow fever from which we have had the opportunity of examining satisfactory blood films, we have detected the presence of minute endo-globular bodies

resembling the *Paraplasma flavigenum* of Seidelin; in the accompanying plate (Pl. VI) a number of these bodies is illustrated. In some cases they were very scarce, but in others they were by no means rare. They were, for instance, more common than malarial parasites are in adult natives suffering from mild attacks of malarial fever, in whom it is often extremely difficult to find conclusive evidence of infection, the parasites being sometimes detected only after examining several blood films, or by employing the thick film method.

Figures 5-12, Pl. VI, represent different forms of bodies found on several days in a single case of yellow fever in a native. In this case blood films were obtained daily from the first day of the disease until the twelfth day, when convalescence was apparently complete. It is noteworthy that the bodies were found on eleven out of the twelve days of the illness, and that they persisted after the subsidence of the fever. This fact is not, of course, exceptional for protozoal diseases of a chronic type, although at first sight it seems remarkable in an acute febrile disorder like yellow fever which, moreover, is said to be infective only during the first three days. It should be remembered, however, that, as Seidelin has pointed out, this belief rests on a very flimsy basis of fact, and cannot be considered as yet to have received satisfactory scientific proof. It is possible that the persistence of these bodies in the blood during convalescence may explain some of the obscure features of the epidemiology.

Some other forms of these bodies encountered in the blood of yellow fever patients are illustrated in figures 1-4 and 13-15, Pl. VI. Figures 16-27 represent the bodies found in guinea-pigs, figures 28-39 those found in dogs, and figures 40-44 those found in rats. The earliest form consists of a mere dot of chromatin with a small blue-stained body. In later stages the size of the blue-stained body increases, until a definite ring form is developed. Several stages of this phase are figured. Ultimately a body of relatively considerable size is produced.

TABLE III.—Synopsis of the experiments on animals.

Case			Day of the disease on which inoculations were made	Animal Reactions					
				Guinea-pig		Dog		Rat	
Commission No.	Race	Result		Febrile reaction	*Paraplasma*-like bodies	Febrile reaction	*Paraplasma*-like bodies	Febrile reaction	*Paraplasma*-like bodies
41	Syrian	Death ...	3	−	−	−	−	...	...
42	Kroo-boy	Recovery ...	8	+	+	−	+	...	...
43	Yoruba	,,	6	+	+	−	+	...	...
44	British	,,	8	+	+	...	...	...	...
45	Beni	,,	5	+	+	−	+	...	...
47	Yoruba	,,	3	+	+	...	...	...	...
51	Kroo-boy	,,	2	+	+	...	...	...	...
52	Kroo-boy	,,	2	−	+	...	...	...	...
53	Kroo-boy	,,	2	−	+	...	...	...	...
55	Yoruba	,,	4 or 5	+	+	...	...	−	+
56	Yoruba	,,	1	−	+	−	+	+	+
			1	+	+	...	...	...	...
			1	+	+	...	...	...	...
			6	+	+	...	...	...	...

+ = present ; − = absent.

The general resemblance of these bodies to the *Paraplasma flavigenum* of Seidelin cannot be denied. We have found the same forms, and although we have not been able to make out a definite cycle of development, the types of body illustrated have been constant in our cases. We are aware that Seidelin's bodies have been severely criticised by various authors, but we are, nevertheless, inclined to the belief that they are parasites. We are of opinion that the constancy with which they are found in the blood of yellow fever

patients is a matter of considerable importance, and should be a valuable aid to the diagnosis of doubtful cases. The experiments on animals, of which an account follows, supply in our opinion, confirmation of the parasitic theory. It would be a remarkable coincidence if bodies of this type were found in human blood from a case of yellow fever, and in the blood of guinea-pigs and dogs inoculated with it, were they not specifically related to the disease. As regards the biological nature of these bodies, we agree with Seidelin in regarding them as belonging to the *Babesiidae*. The cycle undergone by the parasite cannot at present be described in detail, several different forms occurring in the blood at the same time, and unfortunately it has been impossible for us to carry out any experiments with infected mosquitos.

Observations on Guinea-Pigs

Thomas (1907) succeeded in producing a reaction in guinea-pigs from four and a half to thirteen days after being bitten by infected *Stegomyia*, and Seidelin (1912) observed intracorpuscular bodies resembling *P. flavigenum* in two guinea-pigs which he had inoculated from a case of yellow fever. We, therefore, decided to attempt the infection of guinea-pigs by inoculation from the yellow fever cases at Lagos.

The details of the experiments were as follows:—

Guinea-pig 49. Inoculated from Case No. 41, a Syrian, on the third day of his disease.

No febrile reaction was observed in this animal during the eighteen days it was under observation, and no *Paraplasma*-like bodies were found in the red blood corpuscles. Phagocytosis of the red cells by what appeared to be large mononuclear leucocytes was, however, observed in this animal.

Guinea-pig 56. Inoculated from Case No. 42, a Kroo-boy, on the eighth day of his disease.

On the third day the guinea-pig's temperature began to rise, reached its maximum on the fourth day, remained somewhat high until the eleventh day, and then fell to normal. *Paraplasma*-like bodies were found in the red blood cells. On the thirty-eighth day the guinea-pig was found dead. On examining the body the liver was seen to be small and of a deep red colour; the gall-bladder was distended; there was some free ascitic fluid; and the kidneys were dark. The other organs appeared to be healthy, and no obvious cause of death was detected. A small quantity of urine from the bladder was found to contain albumin.

On microscopical examination the liver was found to be intensely congested. The kidneys also were congested, many small haemorrhages had taken place, the tubules contained débris, the lining epithelium was swollen and the protoplasm of the cells was granular; the glomeruli were congested, and in some places degenerated.

GUINEA-PIG 63 was injected from guinea-pig 56 on the sixth day. On the following day the guinea-pig had a well-marked febrile reaction and the fever did not ultimately subside until the fourteenth day. *Paraplasma*-like bodies were present in the blood.

GUINEA-PIG 57. Inoculated from Case No. 43, a Yoruba, on the sixth day of his illness.

The guinea-pig showed a well-marked febrile reaction lasting for thirteen days. *Paraplasma*-like bodies were found in the blood. On the twentieth day the guinea-pig died suddenly. On examining the body there was found oedema of the abdominal walls, the abdominal cavity contained a fair quantity of ascitic fluid, the liver was large and the gall-bladder distended, and the kidneys also were increased in size. Some urine obtained from the bladder was found to contain a trace of albumin. On microscopical examination the liver was found to be acutely congested. The kidney was acutely congested, there were some haemorrhages, and the renal cells were swollen.

GUINEA-PIG 58. Inoculated from Case No. 45, a Beni, on the fifth day of his illness. On the following day the guinea-pig had well-marked fever, which fell slowly until it reached a normal level on the thirteenth day. *Paraplasma*-like bodies were found in the blood.

GUINEA-PIG 59. Inoculated from Case No. 44, a European, on the eighth day of his illness.

On the following day there was well-marked fever, which persisted with some irregularity until the eleventh or twelfth day. *Paraplasma*-like bodies were found in the blood.

GUINEA-PIG 64. Inoculated from Case No. 47, a Yoruba, on the third day of his illness.

The temperature of this animal did not begin to rise until the fourth day, and the febrile reaction was never pronounced, but *Paraplasma*-like bodies were found in the blood.

GUINEA-PIG 67. Inoculated from Case No. 51, a Kroo-boy, on the second day of his illness.

The febrile reaction in this animal was but a slight one, the highest point reached being on the fourth day. *Paraplasma*-like bodies were found in the blood.

GUINEA-PIG 69. Inoculated from Case No. 52, a Kroo-boy, on the second day of his illness.

The temperature of this animal never rose above 101·2° F., and it cannot therefore be said to have shown any definite febrile reaction. *Paraplasma*-like bodies were, nevertheless, found in its blood.

GUINEA-PIG 73. Inoculated from Case No. 53, a Kroo-boy, on the second day of his illness.

As in the previous animal, no febrile reaction occurred, but *Paraplasma*-like bodies were found to be present in the blood.

GUINEA-PIG 79. Inoculated from Case No. 55, a Yoruba, on the fourth or fifth day of his illness.

This guinea-pig showed a well-marked febrile reaction, but the temperature seemed to have returned to normal about the seventh or eighth day. *Paraplasma*-like bodies were found in the blood. On the morning of the fifteenth day the guinea-pig appeared to be in good health. A few minutes later it was found lying down as if its legs had given way under it. Within a few minutes it was able to sit up, and presented a curious appearance with its head sunk between its shoulders, and its hair staring. Its temperature was below 95° F. During the course of the day it recovered, and at 5 p.m. its temperature was 100° F. On the following morning the guinea-pig was

found in a dying condition. It died at 8 a.m. On examining the body the organs did not appear to be severely affected. The liver was large and red, the kidneys were congested, and the mucous membrane of the stomach was a little congested. The urine in the bladder was free from albumin. On microscopical examination the liver was found to be congested, the hepatic cells somewhat swollen, and their protoplasm granular. The kidney was congested, a number of small haemorrhages had taken place, the tubules contained a little débris, and their lining epithelium was somewhat swollen. No gross changes were observable in the heart; the protoplasm of the muscle fibres was, however, rather granular.

GUINEA-PIG 92. Inoculated from Case No. 56, a Yoruba, on the first day of his illnes.

On the fourth day the guinea-pig showed a very slight rise of temperature. A few *Paraplasma*-like bodies were found in the blood, and phagocytosis of the red blood corpuscles was seen to have occurred. On the sixth day the guinea-pig was killed for the purpose of examining its organs. On opening the body the liver was found to be large and of a dark colour. No obvious morbid conditions were present. On microscopical examination the liver was found to be congested, the hepatic cells were granular and appeared to be degenerated. The kidney was congested, the tubules contained débris, and the lining epithelium was swollen and granular. The lungs were congested and their alveoli contained some débris.

GUINEA-PIG 94. Inoculated from Case No. 56, a Yoruba, on the first day of his illness.

This guinea-pig showed a moderate febrile reaction, and *Paraplasma*-like bodies were found in the blood.

GUINEA-PIG 95. Inoculated from Case No. 56, a Yoruba, on the first day of his illness.

This guinea-pig showed a moderate febrile reaction, and *Paraplasma*-like bodies were found in the blood.

GUINEA-PIG 103. Inoculated from Case No. 56, a Yoruba, on the sixth day of his illness.

The animal showed a definite but somewhat irregular febrile reaction, and *Paraplasma*-like bodies were found in its blood.

Fourteen guinea-pigs were thus inoculated from eleven cases. A single animal was injected from each of the first ten cases, and four animals from the eleventh. In each case only one or two drops of blood diluted with 1 per cent. sodium citrate solution were used for the injection, which was made subcutaneously. Ten of the animals showed a definite febrile reaction. Three guinea-pigs that were inoculated in a similar manner with normal human blood did not show any such reaction. Positive results have been obtained by inoculations made as late as the eighth day of the disease. In Table 3 particulars of the experiments will be found; and the four temperature charts reproduced illustrate the nature of the febrile reaction. The temperatures of all the animals were taken *per rectum* daily at 5 p.m.

Seidelin, in his experiments, observed that there were slight

oscillations of the body temperatures during the first seven days following the injections, whilst distinctly higher temperatures were recorded later. He was doubtful, however, whether this apparent rise could be considered of much importance, as he found considerable variations of the temperature in healthy guinea-pigs, and because he had discovered that sufficient care had not been taken in recording the temperatures of his experimental animals. These objections cannot be raised in the case of our guinea-pigs, which showed a well-defined febrile reaction following inoculation. The guinea-pigs did not appear to suffer from any other symptoms of disease, but it is often difficult to detect signs of illness in these small animals. Three guinea-pigs (Nos. 56, 57, 79), however, died suddenly on the 38th, 20th and 16th day after inoculation respectively. All three had shown a well-marked febrile reaction (see Charts 1, 2 and 3), but their temperatures had returned to normal some time before their deaths occurred. Details of the post-mortem appearances and the pathological conditions of the organs will be found above, but we may state again that in all three the liver was congested, and that the kidneys showed signs of parenchymatous nephritis. In two the urine obtained from the bladder was albuminous. As no other deaths occurred among our stock of guinea-pigs at the same time, it would appear probable that the fatal results in these three animals must be attributed to the effects produced in them by the inoculations with yellow fever blood.

Four of the guinea-pigs inoculated failed to show any definite febrile reaction. In two of these, at any rate, auto-erythro-phagocytosis was observed. Elsewhere (1913) we have suggested that the phenomenon of auto-erythro-phagocytosis seems to be peculiarly associated with protozoal diseases. Connal (1912), moreover, has suggested that the ingestion of red cells by macrophages may be a natural reaction of the host to combat an infection. In these guinea-pigs, therefore, the occurrence of this phenomenon may have indicated a successful reaction on the part of the host, and may thus explain the absence of fever,

CHARTS 1 TO 4. TYPICAL CHARTS SHOWING THE FEBRILE REACTION IN INOCULATED GUINEA-PIGS.

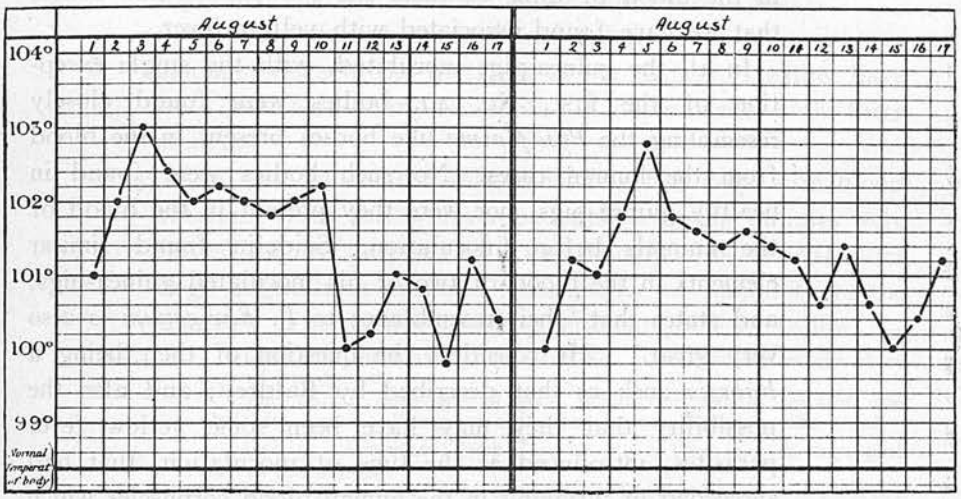

CHART 1. Guinea-pig 56. CHART 2. Guinea-pig 57.

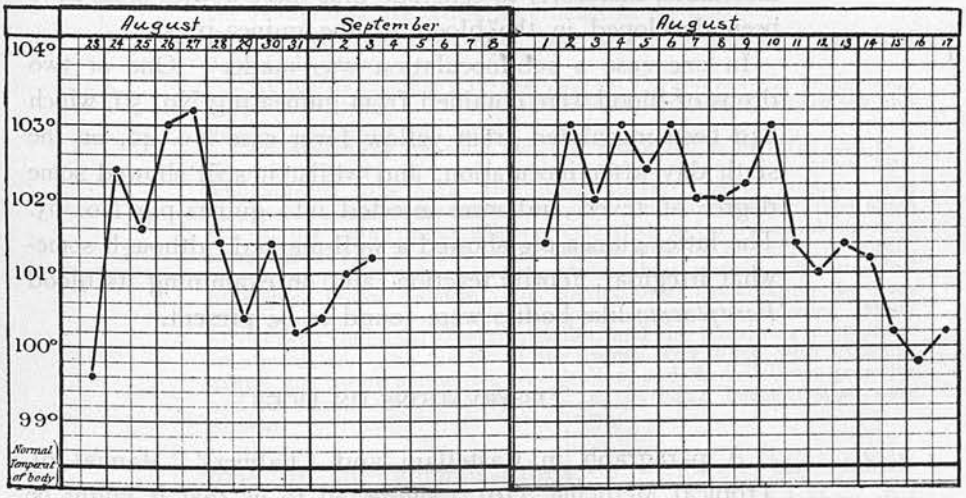

CHART 3. Guinea-pig 79. CHART 4. Guinea-pig 59.

the attacks being, in short, aborted. That the animals were infected, we believe to be proved by the fact that we detected in the blood of three of them the *Paraplasma*-like bodies that we have found associated with yellow fever.

In all the guinea-pigs inoculated, with the single exception of the first, No. 49, bodies were found closely resembling the *Paraplasma*-like bodies present in the blood from the human cases. No such bodies were found in healthy guinea-pigs, nor were they present in the blood of the animals before inoculation. Seidelin found similar elements in the blood of two of his inoculated guinea-pigs, and states that 'their resemblance to *P. flavigenum* is also very great.' He considers the question of their being a *Babesia* such as that described by Baldrey; and also the possibility that they may have been some yellow fever parasites, introduced at the time of inoculation, that had succeeded in surviving in the human blood corpuscles which had been injected. The latter consideration cannot apply in our cases, as the quantity of human blood injected was in every case exceedingly small, and was not introduced directly into the blood stream of the animals. It seems inevitable, therefore, to conclude that these bodies must have been developed in the blood of the guinea-pigs.

In one case a sub-inoculation was made. One or two drops of blood were obtained from guinea-pig No. 56, which had been inoculated from yellow fever case No. 42, on the sixth day after inoculation, and whilst it still showed some degree of fever, and were injected into guinea-pig No. 63. The latter guinea-pig showed a well-marked, although somewhat irregular, febrile reaction, and on examining its blood *Paraplasma*-like bodies were found to be present.

Observations on Dogs

A paragraph in Castellani and Chalmers' 'Manual of Tropical Medicine' (1913) suggested to us that it might be of some interest to perform some experiments with dogs. These authors wrote: 'It is well to remember that in yellow

fever epidemics it is stated that dogs and fowls are supposed to be ill, but from what cause is unknown. Manson has suggested that the disease may be kept up by animals.'

We have failed to find any reference to this sickness amongst dogs in the literature at our disposal; neither have we come across any accounts of experiments with these animals.

We have inoculated subcutaneously five dogs with blood obtained from yellow fever patients. Young puppies were chosen for this purpose, as it was thought possible that older dogs might have acquired some degree of immunity to the disease. The details of the experiments were as follows:—

Dog 50. Inoculated from Case No. 41, a Syrian, on the third day of his disease.

The puppy remained in good health, no febrile reaction was observed, and no *Paraplasma*-like bodies were found in the blood.

Dog 53. Inoculated from Case No. 42, a Kroo-boy, on the eighth day of his illness

The dog remained in good health, no febrile reaction was observed, but *Paraplasma*-like bodies were found to be present in the blood.

Dog 54. Inoculated from Case No. 43, a Yoruba, on the sixth day of his illness.

The dog remained in good health, no febrile reaction was observed, but *Paraplasma*-like bodies were found in the blood.

Dog 55. Inoculated from Case No. 45, a Beni, on the fifth day of his illness.

The dog remained in good health, no febrile reaction was observed, but *Paraplasma*-like bodies were found in the blood.

Dog 96. Inoculated from Case No. 56, a Yoruba, on the first day of his illness.

No febrile reaction was observed, but *Paraplasma*-like bodies were found in the blood. The dog, unfortunately, developed distemper and mange, and was killed on the fifth day.

On examining the body the liver was found to be large and of a deep red colour. The gall bladder was distended with green bile. The kidneys were dark; the spleen appeared to be normal. The mesenteric glands were much enlarged. The stomach contained much mucus, the lining membrane was catarrhal and a few pinkish spots were observed on it. The small intestine was full of parasitic worms. The lower part of the gut was free from these parasites, but was full of a dark brown material. On microscopical examination the liver cells, practically throughout the organ, contained fat globules. Many of the cells contained several globules, and the remaining protoplasm was granular. The tubules of the kidney were filled with granular débris, the lining epithelium was swollen, and the cells were granular and in some places contained fat. A few small haemorrhages were present.

None of the animals, therefore, showed any febrile reaction, their temperatures remained steady, and they did not appear to suffer any symptoms as a result of the inoculations. Nevertheless, on examining blood films, *Paraplasma-*

like bodies were detected in four out of the five puppies. The pathological conditions found at the examination of Dog 96 were probably the result of intercurrent disease, but it is impossible to be certain whether or not they were entirely so.

We have also examined eleven stray dogs from Lagos that had been procured for us by the police. In the blood of two of these *Babesia*-like, or *Paraplasma*-like bodies were found.* One of these dogs, No. 81, was of considerable interest. Dr. Kâpo, in forwarding to us the blood films, reported that the dog was in a dying condition when he saw it; and Dr. Leonard further informed us that the animal, which had meanwhile been destroyed, as it appeared to be in great pain, had had convulsions, and had passed some tarry faeces. Dr. Leonard very kindly made a post-mortem examination, and forwarded to us a kidney, a piece of the liver, and some urine from the bladder. With regard to the post-mortem appearances, he stated that there was no obvious cause of death, but that the liver appeared to be fatty, and that the bowel was full of a dark material. On examination, the urine was found to be acid, and to contain albumin; the liver was congested, and a few of the hepatic cells contained fat globules; and in the kidney the tubular epithelium was swollen, the tubules were full of granular débris, the cells in some places had undergone degeneration, and the blood vessels were engorged. In the blood films taken shortly before the animal was destroyed, a number of intracorpuscular bodies showing well-defined red granules and distinct blue-stained bodies were found (Plate VI, figs. 36, 38). None of the bodies corresponded to the type of *Babesia canis*, but one (Pl. VI, fig. 39) appeared, by its large size, to differ from *Paraplasma flavigenum*, and it is possible that an infection with another species of *Babesia* may have been present.

The histological condition of the liver of this dog was very different from that found in such diseases as distemper, in which fatty degeneration occurs. The first disease that suggested itself as having been the cause of the illness was of course, babesiasis. The post-mortem appearances in

* Similar *Babesiae*, resembling *Paraplasma flavigenum*, have been found by one of us (J.W.S.M.) to be common in sheep and cattle also.

animals that have died from *Babesia canis* are, however, different from those met with in this case, and the histological lesions are dissimilar. In babesiasis the central vein and the interlobular capillaries of the liver are much dilated, and also the intralobular vessels. The fibrous tissue is normal, but the liver cells are distorted, and in many cases destroyed. The kidneys only show dilatation of the blood vessels.

It is, perhaps, too much to suppose that this dog suffered from yellow fever, but in some respects the pathological conditions suggested this disease, and the occurrence of a few *Babesia*-like bodies in the red blood corpuscles, which were very like the *Paraplasma*-bodies found in the dogs inoculated from cases of yellow fever, may be considered to support this hypothesis.

Our observations on this point have been too few and too incomplete to justify any conclusions. The two dogs referred to certainly appeared to be naturally infected with a *Babesia* resembling the *Paraplasma flavigenum*. It is, of course, possible that the parasite was of another nature; but so far as we are aware, no *Babesia* of dogs has hitherto been described presenting the appearance of that observed by us.

The experiments on puppies, already referred to, further suggest that it might be possible for dogs to be naturally infected with yellow fever, and, although harbouring the parasite in their blood, to exhibit no symptoms of the disease. In this way they might conceivably become a reservoir of yellow fever, and the disease might be maintained in them.

Observations on Rats

A stock of white rats having reached us from England, we were able to inoculate these animals from two of the last cases of yellow fever.

Rat 78. Inoculated from Case No. 55, a Yoruba, on the fourth or fifth day of his illness.

The animal showed no febrile reaction, and did not appear to be in any way affected by the injection. *Paraplasma*-like bodies were, however, found in the blood.

Rat 92. Inoculated from Case No. 56, a Yoruba, on the first day of his illness.

On the fourth and fifth days the animal showed some degree of fever, and *Paraplasma*-like bodies were found in the blood.

These two experiments, as far as they go, tend to show that rats are less susceptible than guinea-pigs to inoculation with blood from yellow fever patients. The temperature chart of the one rat that appeared to show some reaction is reproduced below (Chart 5) for comparison with the reaction in guinea-pigs.

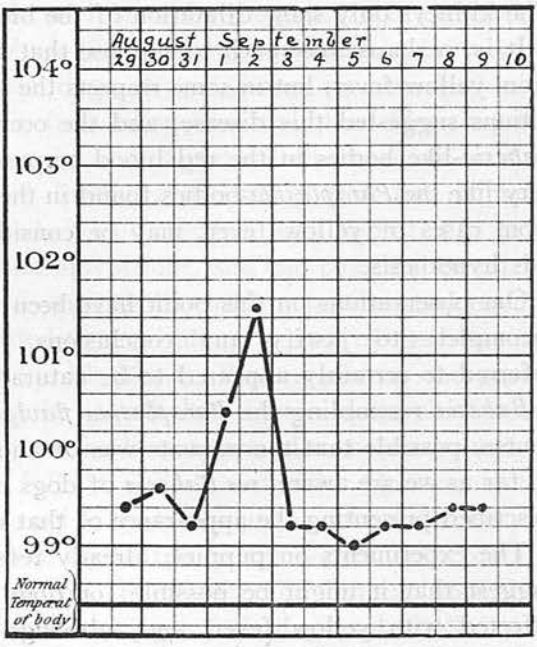

CHART 5. Rat 93.

OBSERVATIONS ON FOWLS

The statement made by Castellani and Chalmers, referred to above, to the effect that fowls are supposed to be ill during epidemics of yellow fever, led us to examine a few birds for the presence of *Paraplasma*-like bodies in the blood. For this purpose six fowls were bought at one of the Lagos markets. They were selected at random, and were a somewhat poor-looking lot. The examination of their bloods was negative.

No disease of fowls in the least suggestive of yellow fever has been reported to us during the recent outbreak of yellow fever in Lagos. The only illness that has come under our notice during this time has been spirochaetosis.

Miscellaneous Observations

The action of Atoxyl. It has been suggested, naturally, that Atoxyl, which has proved so beneficial in certain other protozoal diseases, might also be of value in the treatment of yellow fever. On account of the toxic properties of this drug some reluctance might be felt in employing it in human cases, and it is, therefore, of the utmost importance to carry out therapeutic experiments with it in animals. It occurred to us that, as we had obtained febrile reactions in guinea-pigs following inoculation with the blood from yellow fever patients, it might be possible to test the efficacy of this drug on them.

Two guinea-pigs (Nos. 94 and 95) were, therefore, inoculated with blood from the yellow fever case No. 56, on the first day of the disease. On the fifth day, both animals showed a well-marked febrile reaction. One of them, No. 94, was then injected subcutaneously with 1/50 of a grain of Atoxyl. No marked difference was observable in the subsequent course of the fever in these two animals. In each of them the temperature gradually returned to normal. The temperature charts are reproduced below for comparison.

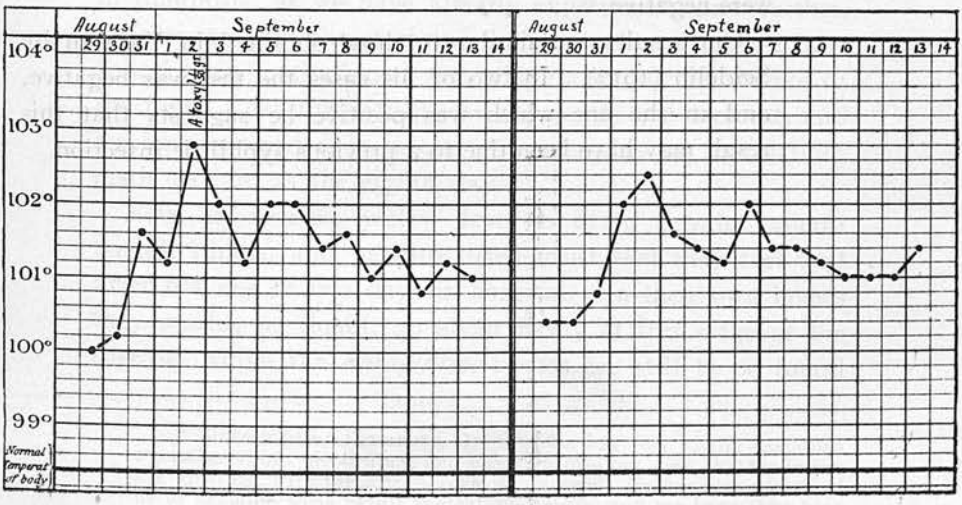

Chart 6. Guinea-pig 94.

Chart 7. Guinea-pig 95.

Unfortunately for our purposes, this was the last case of yellow fever from which we were able to perform experiments, and we were, therefore, unable to make further observations on the action of Atoxyl, and, as we had hoped to, on that of salvarsan. We had also contemplated trying the effect of trypan blue, which has been found by Nuttall and Hadwen (1909) to be a specific in canine babesiasis.

Cultivation experiment. The success of Bass and subsequent workers in cultivating the malarial parasite and the *Babesia* suggested that, if yellow fever were due to an infection with a *Babesia*-like organism, it might be possible to demonstrate it by this means.

In one case we were able to obtain sufficient blood to attempt cultivation. The technique observed was that described by Thomson, McLellan and Ross (1912). The experiment was unsuccessful, and no growth of protozoal organisms was observed.

Wassermann reaction. In two cases the Wassermann test was carried out. The technique employed was that described by Fleming (1909). In the one it was tried on the fifth day, but in the other, unfortunately, it was impossible to attempt it before the tenth day of the illness. The observations are too few to be of any value, but it may be recorded that both were negative.

Our results are similar to those obtained in Yucatan by Seidelin (1912). In two of his cases the test was negative, and in the one which was positive he suggests that this result may have been due to a previous syphilitic infection.

Summary

In 1909, Seidelin described bodies in the blood of yellow fever patients which he believed to be the parasite of the disease, and he has since published a series of papers on the same subject. As far as we are aware, his observations have so far only been confirmed by Hernandez (1912).

On commencing our investigations at Lagos we were first at a disadvantage, as we had not seen specimens of these bodies. It was not long, however, before we discovered in the red corpuscles bodies which we believed to be identical with the *Paraplasma flavigenum* of Seidelin.* We have found these elements in practically every case of yellow fever we have examined, and also in guinea-pigs, dogs, and rats that had been inoculated from human cases. We have found guinea-pigs the most susceptible of the animals with which we have experimented, and we have succeeded in conveying the *Paraplasma*-bodies by sub-inoculation. The crucial experiment of reinfecting man by inoculating with the blood of an infected guinea-pig, we have not been able to perform.

These *Paraplasma*-bodies are by no means always scanty. They are as a rule sufficiently numerous to be a valuable aid in diagnosis, as we have already experienced, and further, an assistance in the study of yellow fever. Thus we have found them in the blood some days after the subsidence of the fever, when the patient was apparently convalescent, and we have succeeded in infecting guinea-pigs by inoculations made as late as the eighth day.

Now, in natives, yellow fever is often a mild, almost trivial, illness, and patients often insist that they feel well after but one or two days in hospital. In their own homes they would, no doubt, go about freely at this stage of the disease, whilst the *Paraplasma*-bodies are still to be found

* Since writing the above, Dr. Seidelin has very kindly examined a number of our blood films, and has confirmed our view that the bodies are identical with the *Paraplasma flavigenum*. He has also permitted us to examine some of his typical specimens, and we have thus been confirmed in our opinion stated above.

in the blood. If, as we believe, these bodies are the parasites of yellow fever, these patients should be capable of infecting mosquitos and of further spreading the disease. In other words, the patient would be infectious for longer than the generally-ascribed period of three days.

The successful inoculation of dogs and rats suggests a possible subsidiary means by which the disease may be kept endemic, in spite of stringent sanitary regulations.

Moreover, the successful inoculation of laboratory animals opens the way to an exact study of the aetiology of yellow fever, of the parasite of the disease and its cycle in its vertebrate and invertebrate hosts, and of the therapeutic effects of various drugs.

We wish to express our sincere thanks to Dr. T. Russell Leonard for permitting us to make use of his observations on the cases under his care, and for inoculating for us some of the animals. We have also great pleasure in acknowledging our indebtedness to Sergt. F. G. Phipps, R.A.M.C., for the careful way in which he has looked after our experimental animals, and for his invaluable assistance throughout our investigations, and to Miss Mabel Rhodes, of the Lister Institute, for her very skilful drawings.

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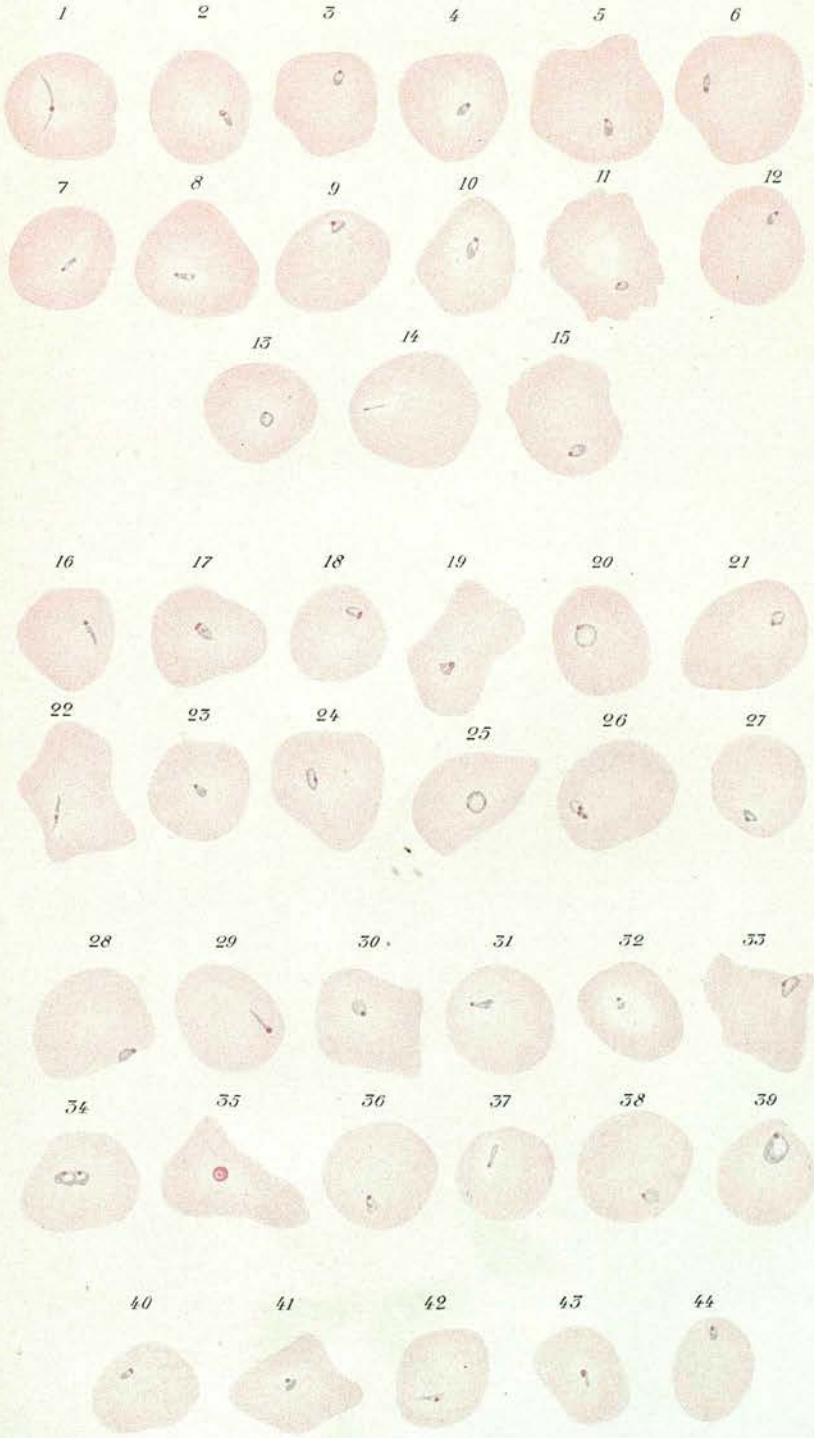
EXPLANATION OF PLATE VI.

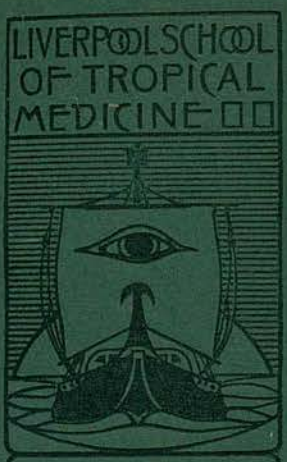
Paraplasma-like bodies found in the blood of yellow fever patients, and inoculated guinea-pigs, dogs, and rats.

Fig. 1.	Case	...	...	11. 5.8.13.
Fig. 2.	,,	...	...	3. 21.7.13.
Fig. 3.	,,	...	...	(Forcados Case.)
Fig. 4.	,,	...	...	6. 31.7.13.
Fig. 5.	,,	...	...	16. 2.9.13.
Fig. 6.	,,	...	...	16. 8.9.13.
Fig. 7.	,,	...	...	16. 30.8.13 .
Fig. 8.	,,	...	...	16. 1.9.13.
Fig. 9.	,,	...	...	16. 7.9.13.
Fig. 10.	,,	...	...	16. 31.8.13.
Fig. 11.	,,	...	...	16. 9.9.13.
Fig. 12.	,,	...	...	16. 30.8.13.
Fig. 13.	,,	...	...	12B. 15.8.13.
Fig. 14.	,,	...	...	Agu. 2nd day.
Fig. 15.	,,	...	...	14. 19.8.13.
Fig. 16.	Guinea-pig		...	73. 28.8.13.
Fig. 17.	,,		...	92. 31.8.13.
Fig. 18.	,,		...	63. 8.8.13.
Fig. 19.	,,		...	103. 8.9.13.
Fig. 20.	,,		...	94. 2.9.13.
Fig. 21.	,,		...	63. 9.8.13.
Fig. 22.	,,		...	63. 9.8.13.
Fig. 23.	,,		...	79. 24.8.13.
Fig. 24.	,,		...	57. 4.8.13.
Fig. 25.	,,		...	92. 31.8.13.
Fig. 26.	,,		...	129. 29.9.13.
Fig. 27.	,,		...	58. 8.8.13.
Fig. 28.	Dog	...	...	53. 2.8.13.
Fig. 29.	,,	...	...	53. 2.8.13.
Fig. 30.	,,	...	...	54. 4.8.13.
Fig. 31.	,,	...	...	96. 3.9.13.
Fig. 32.	,,	...	...	55. 4.8.13.
Fig. 33.	,,	...	...	96. 3.9.13.
Fig. 34.	,,	...	...	96. 3.9.13.
Fig. 35.	,,	...	...	70. 14.8.13.
Fig. 36.	,,	...	...	81. 22.8.13.
Fig. 37.	,,	...	...	70. 28.8.13.
Fig. 38.	,,	...	...	81. Aug., 1913.
Fig. 39.	,,	...	...	81. 22.8.13.
Fig. 40.	Rat	...	...	78. 27.8.13.
Fig. 41.	,,	...	...	78. 27.8.13.
Fig. 42.	,,	...	...	78. 29.8.13.
Fig. 43.	,,	...	...	93. 4.9.13.
Fig. 44.	,,	...	...	93. 2.9.13.



M. Rhodes pinx.

Firenze, lit. A. Puffoni Piazza S. Croce 20



Notes on some Blood Parasites collected in Nigeria

J. W. SCOTT MACFIE, M.A., B.Sc.

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NOTES ON SOME BLOOD PARASITES COLLECTED IN NIGERIA

BY

J. W. SCOTT MACFIE, M.A., B.Sc.

MEDICAL RESEARCH INSTITUTE, LAGOS, SOUTHERN NIGERIA

(*Received for publication* 14 *April*, 1914)

PLATES XXIV, XXV

In the following pages a few notes on blood parasites collected in Nigeria are brought together, and with them is included for want of a better opportunity, the description of a spirochaete found in the gut of a tsetse fly. Individually they would, perhaps, have been too inconsiderable to record; and had it been possible further experiments and observations would have been made in every case.

The paper is arranged in the following sections:—

1. Preliminary note on the occurrence of babesiasis of domestic animals in Nigeria.
2. A spirochaete isolated from the blood of a guinea-pig.
3. Bacilliform bodies found in the red corpuscles of a rat.
4. A disease of fowls characterised by inclusions in the leucocytes.
5. Blood parasites of lizards and toads.
6. The occurrence of a spirochaete in the gut of *Glossina tachinoides*.

PRELIMINARY NOTE ON THE OCCURRENCE OF BABESIASIS OF DOMESTIC ANIMALS IN NIGERIA

Of the diseases of domestic animals in Nigeria considerable attention has been paid to trypanosomiasis, but so far but few observations have been made on babesiasis, and such as have been recorded have related mainly to the disease in dogs. No disease of cattle characterised by haemoglobinuria appears hitherto to have

been reported, and for this reason perhaps the presence of babesiasis in these animals seems to have been entirely overlooked. The insignificant size of the parasites, moreover, which would easily escape detection unless especially searched for, may account for the scant attention paid to them.

In August, September, and October, 1913, blood films were obtained from the slaughter-houses at Calabar, Warri, and Onitsha, and a few were also collected at Lagos. Altogether fifty-nine animals were examined, fifteen goats, seventeen Hausa cattle, two dwarf cattle, and twenty-five sheep. In fifteen, two Hausa cattle, two dwarf cattle, and eleven sheep, babesias were detected; that is over 25 % of the animals were found to be infected. As some of the blood films were unsuitable for examination for minute parasites, it is possible that the percentage should be placed even higher than this.

TABLE 1.—The results of 59 examinations of domestic animals in Nigeria for the occurrence of babesiasis.

Station	Examined				Number infected with babesiasis
	Goats	Sheep	Hausa cattle	Dwarf cattle	
Calabar	12	...	17	...	2 Hausa cattle
Ikotobo	...	...	...	2	2 Dwarf cattle
Lagos	...	5	...	...	4 sheep
Onitsha	3	9	...	...	7 sheep
Warri	...	11	...	...	None
Totals ...	15	25	17	2	15

In the majority of the cases the parasites were numerous, but in three apparently healthy sheep examined at Lagos they were scanty. One of these sheep had been under close observation for two months before its blood was examined, and during this time had never shown any signs of illness, neither did it appear to be unwell at the time of examination, nor during the succeeding four months. Haemoglobinuria was never observed. The other two sheep were

found living in a compound in Lagos in which a case of yellow fever had occurred. They appeared to be perfectly healthy animals, but on examination a few minute ring-shaped babesias were found in the red blood corpuscles (Pl. XXIV, figs. 1 and 2). The close resemblance of these parasites to the *Paraplasma flavigenum* of yellow fever is remarkable, but need not be discussed here.

The majority of the cases of babesiasis occurred in animals that were about to be slaughtered, and no evidence was available to show whether the disease was accompanied by severe symptoms or not. The infected animals examined at Lagos were healthy, but in them the parasites were rare. A somewhat larger infection in cows of the dwarf breed has recently been observed at Ikotobo. The animals were in splendid condition, and their urine was free from haemoglobin. In some of the sheep and cattle examined at Onitsha and Calabar considerable numbers of parasites were found. The animals in these cases had been imported into Southern Nigeria from the north. It is unlikely that such heavy infections could have been entirely benign.

So far as could be judged from the material available the babesias in all these animals, with two exceptions to be referred to later, were of the same species. The parasites were minute, seldom exceeding 1 μ to 1·5 μ in their longest diameters. They were pleomorphic. In stained preparations the most common forms were rounded, pyriform, or rod-shaped bodies. The chromatin as a rule consisted of a single minute dot at one end of the parasite, crescent-shaped, or horseshoe-shaped masses were, however, not infrequent, and occasionally two small dots were seen situated at the extremities of a diameter in the rounded and ovoid forms. In all the cases in which the parasites were numerous what appeared to be multiplication forms occurred, which consisted of groups of four chromatin grains arranged so as to form a cross.

The morphology of this parasite corresponds with the description given of *Theileria parva*. It should be mentioned, however, that Koch's blue bodies were never found, but as these are only occasionally seen in the peripheral circulation, this observation is not of much significance. Theiler, however, has described by the name *Piroplasma mutans* a minute parasite in cattle in South Africa and

Madagascar which resembles *Th. parva* but for the absence of Koch's blue bodies, and the innocuous nature of the infection. It is possible, therefore, that the parasites found in Nigeria may have belonged to this species, although they appeared on the whole to be smaller.

An attempt was made to cultivate this parasite after the manner of Bass. For this purpose blood was obtained from a ram by venipuncture, and transferred to a tube containing the glucose. The blood, after being defibrinated, was distributed into eight smaller tubes, and then incubated at 37° C. Inadvertently a rather stronger percentage of glucose was employed than that recommended by Bass; 1/10 c.cm. of a 50% solution being added to each 8 c.cm., instead of to each 10 c.cm. of blood.

In the blood of the ram, just before the experiment was started, parasites were fairly numerous. The great majority were simple forms, either rods, rings, or pear-shaped bodies, and dividing forms were rare, and did not exceed 4%.

After *six hours'* incubation the number of dividing forms had risen to 19%, and the simple forms appeared to have grown rather larger. Of the dividing forms 9% showed two chromatin dots, 2% three, and 8% four. After *24 hours* the percentage of dividing forms was 21%, but no forms showing more than four chromatin dots were observed. The parasites continued to be well shaped and numerous up to *90 hours*, and the percentage of dividing forms remained high. After *114 hours* the parasites appeared to be much rarer, and the number of dividing forms was considerably less. Many of the parasites, however, still looked healthy. No forms with four chromatin dots were seen at this stage, but on the other hand a considerable number of naked grains like anaplasmas were found in the red cells. From this time onwards the parasites gradually died out in the culture tubes. Flagellated forms were never observed.

In this experiment, although there appeared to be a definite increase in the number of dividing forms in the culture tubes, no stages of development were observed that had not a counterpart in the blood of the ram before incubation. In the tubes the dividing forms at first appeared as compact groups in the red cells (Pl. XXIV, figs. 23 and 26), but later on the chromatin dots became more and

more widely separated (Pl. XXIV, figs. 24, 25, 27, 28). Finally very few typical groups persisted, but many of the red cells were found to contain single chromatin dots (Pl. XXIV, fig. 29).

It would appear therefore that, as figured by Nuttall (1913),[1] cross forms, consisting of four chromatin grains, probably represent the stage of maximum division of the parasite.

In addition to the *Theileria* described above, one of the Hausa cattle examined at Calabar was found to be infected with a *Babesia*. The parasites (Pl. XXIV, figs. 30-37) were of a considerable size, and occupied a large part of the cells they infected. The red corpuscles in which they were contained appeared to be somewhat shrunken, and stained more intensely than the uninfected cells. The smallest forms, which were sometimes extracorpuscular, consisted of a large chromatin mass, and a small, almost solid, protoplasmic body. More mature parasites showed a large, and often irregularly shaped body, and a considerable amount of chromatin, sometimes divided into several pieces. What appeared to be segmenting forms, composed as a rule of six or seven chromatin masses, were not uncommon (Pl. XXIV, figs. 36 and 37).

Unfortunately only two imperfect blood films from this animal were available for study, but in these the morphology of this *Babesia* did not seem to correspond with that of any species hitherto described. It is possible, therefore, that it should be regarded as a new species.

One of the dwarf cattle also had a double infection. Besides the minute parasites described above, and provisionally identified as *Theileria parva*, a very few larger forms were found (Pl. XXIV, figs. 38-40). One corpuscle contained two large pear-shaped bodies that occupied the greater part of the cell, so that it is probable that this parasite was *Babesia bigemina;* the disease in this instance occurring in its benign form.

In the greater part of Northern Nigeria horses and cattle are plentiful, as well as the smaller domestic animals. The cattle are of the large humped-back or zebu breed, and are generally referred to as Hausa or Fulani cattle. In Southern Nigeria, on the other hand, horses can only be kept in a few places, and the only cattle to be found are of a dwarf breed. The reason for this difference has been attributed to the denser nature of the vegetation in the south,

and the greater prevalence of tsetse flies and the accompanying trypanosomiases. This is no doubt in the main correct, but the observations recorded above suggest that *babesiasis* may be a subsidiary factor.

Southern Nigeria, being without suitable cattle, is largely dependent on Northern Nigeria for its meat supply. In the dry season immense herds of cattle trek south from the provinces of the north. It is well known that but a small proportion of the animals that set out on this journey eventually reach the coast towns. The majority sicken on the road, and have to be slaughtered.

In a former paper (1913)[2] an account was given of the examination of cattle treking through Ilorin on their way to the coast, in which it was shown that very many of them were suffering from trypanosomiasis. The herdsmen are quick to note the first signs of sickness in their stock, and on detecting them at once kill the animals and dispose of the meat at the best price they can obtain. Considerable loss is undoubtedly entailed, as the animals have often to be slaughtered in unfavourable markets.

The blood films obtained from the slaughter-houses at Onitsha and Calabar were from animals that had been bred in Northern Nigeria, and had treked south from that country. Those animals slaughtered at Calabar had, of course, reached the end of their journey, but those killed at Onitsha had not. It is improbable that the latter would have been sacrificed had they not been showing indubitable signs of illness, and the large number of babesias found in their blood suggests that in them these parasites were the cause of a serious disease. Since apparently healthy domestic animals in Southern Nigeria have been found infected with babesias, it is probable that the imported animals from Northern Nigeria became infected in this country, and not possessing the natural immunity of the indigenous stock, were seriously affected.

From time to time it has been suggested to improve the breed of various domestic animals in Nigeria by the introduction of European stock. In any such experiments it will be necessary to guard not only against the tsetse fly that transmits trypanosomiasis, but also against the tick that transmits babesiasis.

BABESIASIS OF GUINEA-PIGS

In this place brief mention should be made of two *babesia*-like parasites occurring in guinea-pigs. Their pathological significance is at present quite obscure, and several animals known to be infected with one or other of them have remained in apparent good health for many months. It should be pointed out, however, that in Nigeria guinea-pigs are liable to die suddenly without apparent cause, and that it is possible that in some of these cases death may be due to the parasites described below. The importance of these bodies is enhanced in view of the extensive use that has recently been made of guinea-pigs in studies relating to yellow fever, as it is necessary to exclude their presence before making use of the animals for experimental purposes.

Paraplasma flavigenum (Pl. XXIV, figs. 41-43).

The first was a parasite, closely resembling some of the forms of *Theileria parva*, which was found in two stock guinea-pigs that had come from Calabar and Aro respectively.

Morphologically it was indistinguishable from *Paraplasma flavigenum* as it appears in the human blood, and in guinea-pigs inoculated from persons suffering from yellow fever. The parasites, as they appeared in the red corpuscles, were composed of a minute chromatin dot, and a rounded or oval protoplasmic body. The protoplasm often enclosed a vacuole, but more minute forms with solid bodies also occurred. In some cases the chromatin was divided into two pieces, but with this exception no other stages of division were seen in the two cases studied. In both the parasites were always rare.

Considering the endemicity of yellow fever in Nigeria, and the probable frequency of the disease in the natives, it is not impossible that these bodies may actually have been due to a natural infection with this disease. The evidence at present available is insufficient to decide this point with certainty; but if these parasites are not in fact *Paraplasma flavigenum*, they must belong to a new species of that genus very closely allied to it.

Paraplasma cobayae, sp. nov. (Pl. XXIV, figs. 44-49).

The second was a parasite morphologically somewhat similar to the first, but differing from it especially in producing stippling of the red corpuscles. It was found frequently in the blood of stock guinea-pigs obtained from Calabar and Aro. Nothing is known as to its pathological significance, but the most heavily infected guinea-pig met with remained healthy during the five months it was under observation, and twice successfully resisted infection after inoculation with human trypanosomes. In this animal the parasites were almost constantly present in the blood, but on some days they were rare, whilst on others they were plentiful.

The youngest form of the parasite that was recognisable consisted of a dot of chromatin, and a minute, solid protoplasmic body. The chromatin was generally greater in amount than occurred in *Paraplasma flavigenum* at a similar stage of development. The largest forms observed were ovoid or ring-shaped, and were definitely vacuolated. All stages between these two types were met with. Occasionally the chromatin was divided; and occasionally it assumed a horseshoe form.

The red corpuscle in which the parasite was enclosed was always more or less stippled. The degree of stippling varied considerably, being very coarse in some cells, and almost imperceptible in others. The difference seemed to depend on the intensity of the staining, and not on any stage of the development of the parasite; the more heavily stained specimens showing the coarser stippling.

This parasite, although closely allied to *Paraplasma flavigenum*, is easily distinguished from that species by the stippling of the corpuscle, and by the somewhat larger and coarser structure of the organism itself As it appears to be a common parasite of the guinea-pig in Nigeria, we propose for it the name *Paraplasma cobayae*.

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A SPIROCHAETE ISOLATED FROM THE BLOOD OF A GUINEA-PIG

This spirochaete was observed in a culture of the heart blood of a guinea-pig at Lagos. Two c.cm. of blood were drawn off with aseptic precautions and added to a sterile tube containing 1/10 c.cm. of a 50% aqueous solution of glucose. The blood was then defibrinated, transferred to a smaller sterile tube, and incubated at 37° C.

On the third day, after forty-six hours' incubation, a few small spirochaetes were found in the culture. They rapidly increased in number, so that by the fifty-third hour there were a considerable number present, and by the seventy-ninth hour they were innumerable. From this time, however, they gradually decreased in numbers, and many of those that were present appeared to be degenerating. The central core broke up at first into short pieces, and then into small dots or granules which were seen to be still enclosed in a faintly-stained sheath. By the eighth day all spirochaetes had vanished. The spirochaetes appeared to be developing in the layer of serum overlying the corpuscles.

At first the spirochaetes were all very minute (Pl. XXV, fig. 22), but later much larger forms appeared. In a film taken after fifty-three hours' incubation fifty individuals, taken as they came, were measured. The middle lines of the spirochaetes were drawn with a camera lucida, and these were measured by the tangent line method. The details of the measurements are shown in Table 2. The parasites ranged in length from 2 μ to 9 μ, and the crest of the curve of measurements occurred at 5 μ. Two individuals, however, measured 14 μ. These were double forms, a type which at this stage was just beginning to appear in the culture tube.

At the same period of incubation, fifty-three hours, the number of undulations in fifty individuals was counted (see Table 3). The smallest number noted was two, and the greatest nine; but the great majority of the spirochaetes had three, four, or five undulations.

Twenty-six hours later, that is after seventy-nine hours' incubation, fifty more spirochaetes were drawn and measured. This was the period at which the parasites were most numerous in the culture. The majority of the spirochaetes were small, measuring

2 μ to 3 μ, or even less; but a few very long forms were also present. The latter appeared to be multiple forms, and were probably the result of incomplete divisions. In a film taken at this stage one spirochaete was found which measured 35 μ.

TABLE 2.—The distribution, according to length, of 100 spirochaetes from a guinea-pig.

Age of the culture	Number measured	Length in microns																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
53 hours	50	...	1	3	11	13	10	4	5	1	...	...	...	...	2	...	...	...	...
79 hours	50	...	14	12	5	5	2	3	1	2	2	...	...	2	...	1	...	1	...
Totals ...	100	...	15	15	16	18	12	7	6	3	2	...	...	2	2	1	...	1	...

TABLE 3.—The distribution, according to the number of undulations, of 50 spirochaetes from a guinea-pig.

Age of the culture	Number measured	Number of undulations									
		1	2	3	4	5	6	7	8	9	10
53 hours ...	50	...	3	13	12	11	6	2	1	2	...

Already after seventy-nine hours' incubation the spirochaetes had begun to undergo marked structural changes. The characteristic undulations were becoming indistinct, and individuals showing knots of chromatic material, beading of the core, and granule formation were common. From this time onwards these changes progressed until the culture died out on the eighth day. Latterly the spirochaetes did not show any clear undulations at all, and often consisted simply of a shadowy sheath enveloping a few chromatic granules (Pl. XXV, fig. 33).

The spirochaetes found after fifty-three hours' incubation probably represented the true form of the parasite, which should therefore be considered to measure 2 μ to 9 μ in length, and to have two to six undulations. The longer forms, and the forms with more undulations, were probably double. The extremities of the

spirochaete were usually blunt. When observed with dark ground illumination the spirochaetes were seen to be very active.

Although the spirochaetes rapidly underwent structural changes after the seventy-ninth hour of incubation, they apparently retained their infective power. After 100 hours' incubation a loopful of the serum from the culture was inoculated into the clear fluid that had separated out above the corpuscles in a tube of human blood prepared by Bass' method (i.e., 10 c.cm. of blood to 1/10 c.cm. of a 50% aqueous solution of glucose, defibrinated, and incubated at 37° C.). The human blood in this case was not fresh, but had been prepared and incubated at 37° C. for seven days. The spirochaetes readily multiplied in this medium, and were present in sufficient numbers to be detected after forty-three hours. At this stage they were all very minute, not more than 1 μ to 3 μ in length. After fifty hours these small forms were very numerous, and after sixty-six hours longer forms, and a very few double forms appeared.

The original culture when 150 hours old was used to inoculate a clean guinea-pig, a clean white rat, and three tubes of broth. At this stage the culture contained no normal spirochaetes so far as could be determined, but only forms that had undergone the changes referred to above. All the experiments were negative.

I have to thank Dr. Connal, Director of the Medical Research Institute, Lagos, for very kindly allowing me to make the experiment with the blood of this guinea-pig, which was one of a long series of these animals in which the *Paraplasma flavigenum* of yellow fever had been maintained for several months. The guinea-pig at the time it was killed was in a moribund condition, and was supposed to be dying of yellow fever, and it was in the hope of obtaining a culture of *Paraplasma flavigenum* that the experiment was made. No *Paraplasma* was found in the culture, however, and the appearance of the spirochaetes was quite unanticipated, as the guinea-pig was not known to be infected with these organisms.

Dr. Connal informs me that he had never detected spirochaetes in his routine examinations of the blood of the guinea-pig; and a blood film taken at the time of the experiment showed none of these parasites.

de Gasperi (1912)[3] has also fortuitously isolated a spirochaete, in glucose-lactose gelatine cultures, of the heart blood of a guinea-

pig. His spirochaete measured 9·6 μ to 24 μ in length, and had five, six, or eight undulations. The extremities were pointed. In these respects it differed widely from the spirochaete described above.

As there is no evidence that would suggest an association between this spirochaete and the parasite of yellow fever with which the animal was infected, it must be concluded that it was a specific parasite of the guinea-pig.

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BACILLIFORM BODIES FOUND IN THE RED CORPUSCLES OF A RAT

The curious bodies shown on Pl. XXIV, figs. 50-53, were found in a single brown rat at Ikotobo. The rat when captured was in a moribund condition, and died soon after it was brought to me. A dissection of the body revealed no obvious morbid conditions.

In blood films, stained with Giemsa's solution, a few of the red corpuscles were found with the appearance shown in the figures. They contained a number of rod-shaped bodies, closely resembling bacilli, scattered irregularly in the cells. Some cells showed but a few of these bodies, others were literally packed with them. On minuter examination no special structure could be made out in the bacilliform bodies; they measured roughly 1 μ by 0·25 μ, and stained homogeneously a dark blue or purplish colour. The blood of the rat showed well-marked polychromasia, but it was only in red corpuscles that stained the normal pink colour that these bodies were found. No free forms were seen in the blood, neither did the leucocytes appear to have ingested any of them.

Dr. Connal informs me that Dr. O'Brien found similar bodies in the blood of a giant rat at Accra, on the Gold Coast, and that a note on the subject appeared in the Report of the Accra Laboratory for 1911. I have not had an opportunity of consulting this report, but I understand that Dr. O'Brien observed these bodies in the red cells on several successive occasions, that they gradually increased

in number, and that they appeared to be associated with some symptoms of illness.

It is improbable that these bodies could have been due to a remarkable form of basophilia. Their regular shape, and sharply defined outline did not at all suggest such an explanation; and the fact that they only occurred in the normally stained red cells, and not in the polychromatic ones, seemed to be contrary to this view. It is also unlikely that they were bacilli in, or upon, the red cells, as none were found free in the blood, and there was no evidence of their ingestion by leucocytes. The fact that the bodies occurred in considerable numbers in a few corpuscles, and not evenly distributed in most of the red cells, is also against this view. It seems probable, therefore, that they must have been endoglobular parasites, although I am not acquainted with any protist organism at all resembling them.*

A DISEASE OF FOWLS CHARACTERISED BY INCLUSIONS IN THE LEUCOCYTES

The disease described below was observed in five fowls during the months of November and December, 1913. Four of the cases occurred at Eket, and one at Ikotobo. Shortly before my visit to the district, a considerable number of the fowls at Eket had died of this disease, which was well known to the natives, and was considered to be almost invariably fatal.

The symptoms observed were as follows:—The first indication that the fowl was ill was that it showed a tendency to stand stock-still with tail and head drooping. Later it assumed a typical attitude with its shoulders hunched up, its head sunk, its tail depressed, its feathers ruffled, and its eyelids closed. In this attitude the bird would stand indefinitely if undisturbed. As the disease advanced the wings also drooped, and the bird became

*The above note was written in Nigeria where I had not the opportunity of consulting recent literature. Since returning to England I have consulted the work of various authors, and especially the recent article by Laveran and Marullaz (Bull. Soc. Path. Exot., Vol. VII, p. 240. 1914), and I believe that without doubt the bodies observed by me were the same as those originally described by Graham-Smith, and subsequently named *Grahamella* by Brumpt. It should be mentioned, however, that forms such as those figured by Graham-Smith in his coloured plate were never seen by me.

unsteady, and unable to stand except by separating its legs to an unusual extent. Finally it lay on the ground, making no attempt to move, until death supervened. Diarrhoea was a marked symptom. Anaemia was not, however, constant, and in two of the cases it was noted that the comb was of a deep red hue, and that the blood was unnaturally dark, and almost chocolate coloured.

The disease was exceedingly acute, and death usually occurred within two days. On examination the body was as a rule neither much emaciated nor conspicuously anaemic. The heart was distended, and in one case the pericardium was full of a greenish fluid; the spleen was somewhat enlarged and soft; and the liver was enormous, pale, and friable. There were no necrotic patches on the liver. The gut was normal.

On microscopical examination the liver was found to have undergone profound fatty degeneration, and the spleen also contained a little fat. No spirochaetes were observed in either sections or smears of the liver and spleen.

The general appearance of the fowls at once suggested that they were suffering from spirochaetosis. The dropping of the head and tail, the somnolence, and the diarrhoea were characteristic of this disease; but the absence of marked anaemia, the fact that paralyses were never observed, and the shorter duration of the disease pointed to some other condition. The most careful examination of blood films taken at every stage of the disease, moreover, failed to reveal the presence of spirochaetes. In every case, however, leucocytes were found enclosing chromatic granules and rings of a type that did not occur in the blood of healthy fowls.

The leucocytes in which the granules were observed were big cells with a large and often kidney-shaped nucleus, and a considerable amount of blue-stained cytoplasm. The granules varied in size from minute points to large spherical masses. They were, however, most frequently large rounded or annular bodies, but not infrequently assumed a club-shaped or signet-ring form. They appeared to consist entirely of chromatin, and in no case was any blue cytoplasm observed attached to them. In blood smears in which the enveloping cell had burst, these granules and rings were seen lying freely on the slides (Pl. XXIV, fig. 65). In smears of the liver and spleen the same bodies were found in great

quantities in the cells, but in these organs the granules were often much larger than in the peripheral blood, and so crowded together as to form definite masses (Pl. XXIV, fig. 60).

The red corpuscles also were affected. In the earliest stages of the disease red cells were frequently seen the nuclei of which did not stain the normal deep chromatin tint, but took on a somewhat browner hue with Giemsa's solution. Their cytoplasm was relatively scanty, and tended to assume a brownish colour also. In the latest stages corpuscles of the types illustrated (Pl. XXIV, figs. 55-58), the cytoplasm of which was more or less stippled with brownish or purplish granules, appeared in the blood. It is probable that the dark, almost chocolate colour of the peripheral blood already alluded to was due to the presence of these corpuscles. Similar red cells were seen in smears from the livers and spleens of infected fowls.

In three cases subcutaneous injections with atoxyl were given in the hope that this treatment, which is so successful in the case of spirochaetosis, might prove beneficial. Two of the cases were in the early stage of the disease, and had only just begun to show symptoms. In the blood of the first the chromatic granules described above were present in only a very few leucocytes: this fowl recovered; in the blood of the second the granules were very numerous, and many of the red cells showed the changes already referred to: this fowl died. The third case was moribund at the time of the injection, and died two hours later.

Comparing these results with those obtained with the same drug in spirochaetosis there can be little doubt that its efficacy is much less in this disease. It should be mentioned, however, that a number of fowls living in the same run at Eket as two of the cases were given a prophylactic dose of 3/50 gr. of atoxyl apiece, and that no further cases occurred. It had been anticipated that the disease would spread, as it had done on a former occasion, and kill off the majority of the fowls. It is not certain, nevertheless, that the arrest of the epidemic can properly be attributed to the atoxyl.

No ticks were found on the fowls, but only a few minute red mites on the skin under the wings.

One of the cases was that of a British fowl landed from a

steamer at Oron on November 10th, and taken next day to Eket. This bird was first observed to be ill on December 9th, and died the same day. The incubation period in this instance must therefore have been less than a month.

The disease is inoculable. A healthy fowl, in whose blood neither parasites of any sort, nor leucocytes with granules of the type described above could be found, was inoculated subcutaneously with 5 min. of bood from one of the cases. On the fifth day a considerable number of leucocytes containing the characteristic granules were found in its blood. On the sixth day they were less numerous, on the seventh day they were rare, and on subsequent days none were found. No symptoms of illness were observed. This fowl was a native one, and must have been exposed to natural infection all its life, so that it is quite likely that it possessed some degree of immunity to the disease which would account for the transitory nature of the infection. The subsequent history of the fowl would seem to prove that recovery from an attack confers an immunity, for three weeks later it was reinoculated with blood from another case without result.

With the exception of the granules in the leucocytes no other constant feature that suggested itself as of etiological significance was found in this disease which clinically presented a very definite form, and no organisms were detected in the blood and tissues of the fowls to the action of which the condition might be attributed. For this reason it is suggested that these cell inclusions were the specific cause of the disease; an hypothesis which obtains some support from the fact that they were successfully transmitted by inoculation to a healthy fowl.

BLOOD PARASITES OF LIZARDS AND TOADS

During 1913 a number of lizards and toads were examined with a view to determining the blood parasites they harboured. The species of lizard was *Agama colonorum*, and the toad *Bufo regularis*. Altogether thirty-eight lizards and twenty-eight toads were examined; and of these fifteen and twelve respectively were found to have parasites in their blood. Multiple infections were very common. In the blood of one lizard, for example, two species

of micro-filaria, a trypanosome, a haemogregarine, and a haemocystidium were found.

With the exception of one lizard which came from Ilorin, and eight examined at Ikotobo, all the animals were caught in the grounds of the Medical Research Institute, Lagos.

TABLE 4.—Parasites found in the blood of toads and lizards in Nigeria

	Toad (*Bufo regularis*)	Lizard (*Agama colonorum*)
Number examined.....	28	38
Trypanosomes	4	1
Microfilariae	3	13
Haemogregarines	8	2
Haemocystidia	...	3

LIZARDS (*Agama colonorum*).

Of the thirty-eight lizards examined, fifteen were found to have parasites in their blood. Nine had a single infection, and six had a double or multiple infection. Trypanosomes were found in one individual, micro-filariae in thirteen, haemogregarines in two, and haemocystidia in three. The largest number of parasites was always found in the oldest lizards, the most heavily infected being males whose stunted tails and deformed claws seemed to indicate that they were of a considerable age. So far as could be determined, the lizards did not appear to suffer from the parasitic infections. A number of heavily infected individuals were kept in captivity for several months, and appeared to enjoy good health as long as they were under observation.

Trypanosomes (Pl. XXV, fig. 12).

In a single male obtained from Ilorin, Northern Nigeria, broad stumpy trypanosomes were found in the blood. The parasites were extremely scanty, only one being seen in a fresh preparation, and one in a stained blood film.

In the fresh preparation the trypanosome exhibited rotatory movements, but did not swim actively about the field.

The single individual found in the stained film measured 40 μ in length, and about 11 μ in breadth at its widest part. The morphology of the parasite can best be made out from the accompanying sketch (Pl. XXV, fig. 12). The nucleus, which was indistinct, was situated about the middle of the body; and towards the blunter end of the trypanosome there appeared to be a small blepharoplast. The flagellum was attached to the body by a relatively scanty membrane, and there did not appear to be any free portion, or only a very short one, projecting beyond the body.

No figure of a trypanosome exactly similar to this one has come under our notice, but Todd and Wolbach (1912)[4] have described a form in a lizard of this species from the Gambia which was possibly the same.

Filarial embryos

Filarial embryos were present in the blood of thirteen of the lizards examined. They were of two distinct types, which for convenience we may term the slender, and the stumpy forms. In six lizards both forms were present, and in all these enormous numbers of the embryos were found, so that on observing the fresh blood with the microscope, it seemed to consist of little besides a writhing mass of parasites.

The identity of these filariae has not yet been determined, and it would therefore be superfluous to give a detailed description of the embryos found in the blood. Brief mention should, however, be made of them since they form such a striking feature of the blood picture of the lizards in Nigeria.

Slender type (Pl. XXV, fig. 1).

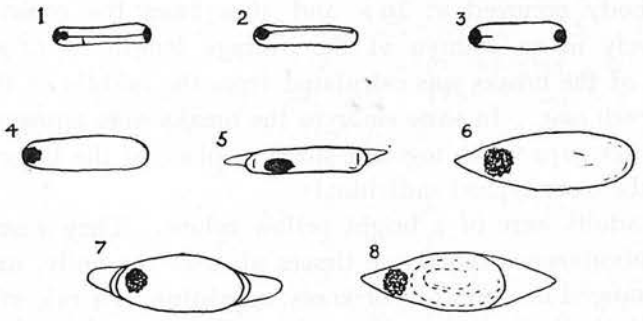
Long, slender filarial embryos were found in twelve lizards. The general form of these parasites is shown in the figure (Pl. XXV, fig. 1). They were enclosed in a sheath which was exceedingly difficult to stain, but within which the embryos could be seen actively moving in fresh preparations of the blood.

The average length of twenty individuals, taken as they came, and roughly drawn with the aid of a camera lucida, and measured by the tangent line method, was 129 μ. The longest was 154 μ,

and the shortest 106 μ. Embryos measuring as much as 168 μ at any rate were occasionally seen. The breadth was about 4 μ at the widest part.

The tail was sharp, but the cephalic end was rounded. There was always a clear area of about 4 μ at the cephalic end. The most constant gaps and breaks in the body occurred, in an embryo of the average length of 129 μ, at 37 μ, 54 μ, 83 μ, 113 μ, and 124 μ from the cephalic end; or in approximate percentages of the total length of the parasite, at 29, 42, 64, 88, and 96 % respectively. As the lengths of the breaks and gaps varied considerably the above figures are calculated to the middle points of the clear areas in each case.

Associated with these micro-filariae, small dumb-bell shaped bodies, about 12 μ long, were invariably found in the blood (Pl. XXV, fig. 4). In intensely stained films these bodies were seen to be surrounded by an irregularly-shaped envelope composed of a material that appeared to be similar to that of the sheaths of the filarial embryos. The association of these bodies with the micro-filariae was not at first suspected. It was noticed, however, that lizards in whose blood at first these bodies alone were seen, later on



were found to be infected with the slender type of filarial embryos, and finally in the uteri of adult filariae from the mesentery both dumb-bell shaped bodies and immature filarial embryos of the slender type were found together. All stages between a simple rod-shaped body with a darker dot at each end and a thin connecting line, and filarial embryos such as appeared in the blood were found. The earlier stages of this development are illustrated by the figures in the text (figs. 1-8), which are sketches drawn with

the aid of a camera lucida from a preparation of the uterine contents of one of the filariae, and arranged in what may be supposed to be their proper sequence.

The adults of these filarial embryos were slender, whitish worms found lying between the folds of the mesentery.

Stumpy type (Pl. XXV, fig. 3).

The stumpy type of embryo was found in seven lizards. It was enclosed within an ample sheath which stained much more easily than that of the slender type. The embryos were relatively short and stout; the tail was pointed, but the cephalic end was somewhat blunt. In fresh films their movements were sluggish. The general form of the parasite is shown in the figure (Pl. XXV, fig. 3). In blood that had been shed for some little time many of the embryos of this type were found to have cast off their sheaths, and to be lying free in the plasma.

The average length of twenty individuals, taken as they came and measured by the tangent line method, was 65 μ. The longest embryo measured 78 μ, and the shortest 56 μ. The average breadth, at the widest part was 5 μ-6 μ. At the cephalic end there was a clear area extending for 3 μ-4μ. The chief breaks in the body occurred at 49 μ and 56 μ from the cephalic end respectively in an embryo of the average length of 65 μ. The position of the breaks was calculated from the middle of the clear area in each case. In some embryos the breaks were represented by two smaller gaps which together stood in place of the larger single gap of the more typical individuals.

The adults were of a bright yellow colour. They were found in the subcutaneous connective tissues all over the body, and were often arranged in close coils or knots, consisting as a rule of a male and a female, in situations where the skin was loose, or were found running down between the muscles of the back on either side of the vertebral column. In the uteri of the females, embryos were seen similar to those found in the peripheral blood of the lizards.

Haemogregarines (Pl. XXV, figs. 16-21).

In the blood of two lizards, the one from Ilorin and the other from Ikotobo, haemogregarines were found.

Both endoglobular and free forms were present. The latter

were rare; but in one of the lizards the former were plentiful. The free forms (Pl. XXV, fig. 20) were rather slender bodies with one end slightly blunter than the other. At this end, lying close to the nucleus, an oval area that stained in a distinctive manner was usually seen to be differentiated in the cytoplasm. The endoglobular forms were enclosed by a definite capsule. They varied in shape from almost spherical bodies to elongated and hoop-shaped forms occupying the whole length, or the whole of one end, of the corpuscles. The corpuscles themselves were not greatly distorted by the parasites, and reacted to staining reagents in a normal manner. The parasites lay within the capsules in a more or less bent position. In the spherical forms they were bent double; but in the more commonly observed elongated forms they lay with their extremities turned only slightly inwards towards the nuclei of the corpuscles.

A considerable number of chromatin granules were always scattered about the endoglobular parasites. They were sometimes fine, and generally distributed, but more often they were coarse and tended to be aggregated in masses at the extremities of the capsules or along their borders. No pigment was present.

An interesting point was the fact that a number of the leucocytes were found to contain haemogregarines (Pl. XXV, fig. 21). The parasites appeared to be enclosed in capsules, and stained similarly to those seen in the red cells. Their appearance did not suggest that they had been ingested and were undergoing a process of digestion.

Parasites of this type, occurring in white cells, have been found in the blood of small mammals and vultures, and have been named *Leucocytogregarina*. The organism described above appears to be peculiar in that it is found both lying freely in the blood, and invading the red and white corpuscles, and would appear to be similar to the drepanidium found by Dutton, Todd, and Tobey in frogs and toads.[5]

Haemocystidia (Pl. XXV, fig. 8).

In three lizards pigmented parasites were found in the red blood corpuscles which should, probably, be assigned to the genus *Haemocystidium* (Pl. XXV, fig. 8). In three other lizards pig-

mented leucocytes were detected, so that it is possible that had more material been available pigmented parasites might also have been found in their blood.

Todd and Wolbach, in the paper referred to above, recorded the occurrence of 'A pigmented haemocytozoon in a lizard (*Agama colonorum*)' which may have been the same parasite; and Wenyon (1908)[6] has described under the name *Haemoproteus agamae* a pigmented parasite in the same species of lizard from Wau, in the Bahr-el-Ghazal. 'The affinities of this parasite,' he says, 'are evidently with those of halteridium, from which it differs, however, in the shape of the gametocytes and, perhaps, in the fineness of the pigment grains.' It is probable that the parasites from Nigeria should be referred to this species.

TOADS (*Bufo regularis*)

Nine of the twenty-eight toads examined were infected with a single species of parasite, and three with two or more. Trypanosomes were found in four, filarial embryos in three, and haemogregarines in eight. A considerable number of the toads examined were quite young animals; but the general impression formed was that the majority of adults is infected with haemogregarines. Trypanosomes and micro-filariae were relatively much rarer, and were always rather uncommon in the blood.

Trypanosomes (Pl. XXV, figs. 10, 11, 13-15).

Three species of trypanosomes were met with in toads, namely, *T. mega*, *T. rotatorium*, and another form which is possibly a new species.

In one toad a trypanosome was found that was apparently identical with *T. mega* (Pl. XXV, fig. 13). The parasite was always scanty in the blood, and in spite of its large size was exceedingly difficult to find. During the first twenty days the animal was under observation, however, it was constantly present in the peripheral blood, and no other forms were ever met with in this animal. The occurrence of this trypanosome in the toad has previously been recorded by several authors.

An interesting observation was made on this parasite. On April 23rd, 1913, the toad was placed in an incubator at a temperature of 38° C. It was thought possible that the unnatural conditions thus produced might enable the trypanosomes to gain

an advantage over the host, and that they might become more abundant in the blood. Forty-two hours later the toad was removed to the ordinary temperature of the laboratory. It appeared to have been adversely affected by the temperature of the incubator, as two blisters had formed, one on either side of its back. On the same day one degenerated trypanosome was found in the blood, but with this exception no trypanosomes were detected during the subsequent 120 days the animal continued to be under observation.

That the trypanosomes did not multiply although the host was obviously affected may have been due either to the effect of the unusually high temperature on the trypanosomes themselves, or to the fact, which has been suggested, that the parasites found in the blood are in a latent stage awaiting an opportunity for further development in another host, but not for the time being undergoing multiplication. In this connexion it is interesting to contrast the experiments of Wendelstadt and Fellmer (1909)[7] on the vitality of mammalian trypanosomes in cold-blooded vertebrates. In recording the results of inoculations with *T. lewisi* they stated that temperature had an influence on the infected cold-blooded animals. Adders and lizards stood *T. lewisi* infection well at room temperature, less well at 37° C. If, as may be supposed, this lowered resistance was due to some diminution in the vitality of the cold-blooded host, the experiment described above with *T. mega* and *Bufo regularis* would seem to indicate that the change of temperature had an even greater prejudicial effect on the vitality of the trypanosome.

Three toads were found to be infected with *T. rotatorium* (Pl. XXV, figs. 10 and 11). The parasites were morphologically identical with those figured in the Fourth Report of the Wellcome Tropical Research Laboratories, and identified there as *T. rotatorium;* and with the figure of *T. sanguinis* from frogs given by Dutton and Todd in their First Report of the Trypanosomiasis Expedition to Senegambia (1902). In only one of the three toads were the parasites at all numerous.

In fresh preparations the ample membrane of the trypanosome was seen to be in constant, and very active motion. The average length including the free portion of the flagellum was 35 μ.

In a single toad, which also harboured *T. rotatorium*, another form of trypanosome was found (Pl. XXV, figs. 14 and 15). This parasite measured from 63 μ to 74 μ in length, and had a long free flagellum. The nucleus was indistinct, the blepharoplast was, on the other hand, large and prominent. The cytoplasm stained almost uniformly blue, but merged insensibly with the membrane, which was ample and deeply folded. The posterior end of the trypanosome was prolonged for a considerable distance beyond the blepharoplast, and gradually tapered to a point. It was not uncommon to find these parasites, in stained preparations, coiled into a ring or spiral.*

The general appearance of the parasite is illustrated in the accompanying plate. We are not aware that any similar trypanosome has previously been recorded from a toad (*Bufo regularis*).

A few attempts were made to transmit *T. mega* and *T. rotatorium* by inoculations. Two lizards were injected, intraperitoneally, with blood containing *T. mega* from the toad. Neither developed trypanosomiasis. Two lizards and four toads were inoculated similarly with toad's blood containing *T. rotatorium*. None of these animals became infected. The lizards remained under observation for twenty-two days, and fifty-four days; and the toads for fifty days, twenty-nine days, and fifty days, respectively.

No dividing forms of any of the above trypanosomes were seen in the peripheral blood.

Filarial embryos

Filarial embryos (Pl. XXV, fig. 2) were observed in three of the twenty-eight toads examined. They were in each case very rare, and it was not possible to find a sufficient number on which to base a study of their morphology. The general form of the parasites is, however, shown in the figure. The length was about 110 μ, and the breadth 4 μ-5 μ. There was no sheath.

The behaviour of these parasites in the circulating blood was watched in the vessels of the mesentery, but beyond the fact that they passed along in very actively wriggling coils, and were never

* Compare Dutton, J. E., Todd, J. L., and Tobey, E. N. 'Concerning certain Parasitic Protozoa observed in Africa,' Annals of Trop. Med. and Parasit., Vol. V, p. 327, and Pl. XXVIII, figs. 43 and 44, showing the coiled forms of a 'trypanosome resembling *T. karyozeukton*.'

seen fully extended, no particular observations were made. Anastomoses between the smaller blood vessels of the mesentery are numerous, and it was not therefore necessary for the microfilariae to enter the minute capillaries. Indeed they were observed on several occasions to be arrested at the mouth of a capillary arising from a larger vessel until a current of blood of sufficient force swept by to carry them along the broader channel.

The adult worms occurred in the mesentery attached to the lower third of the gut.

Haemogregarines (Pl. XXV, figs. 5 and 9, 6 and 7).

Haemogregarines are exceedingly common in the blood of adult toads (*Bufo regularis*) in Nigeria, but they are apparently less frequently present in young animals. The accompanying figures (Pl. XXV, figs. 5, 6, 7, and 9) illustrate the appearance of these parasites. The parasites are large, and occupy a considerable part of the red cells, so that the nucleus is much displaced. Large recurved vermicules are commonly seen, and straight or slightly bent forms of various sizes. In some cases merozoite-like bodies were found free in the plasma. The haemogregarines were enclosed in capsules, at the ends of which small collections of chromatin-staining material were sometimes visible.

The parasite is probably of the same species as that found in *Bufo regularis* in Khartoum, and identified by Stevenson (1911)[8] as *H. tunisiensis*.

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5. Dutton, J. E., Todd, J. L., and Tobey, E. N. Annals Trop. Med. & Parasitol. Vol. I, p. 331.
6. Wenyon, C. M. (1908). Third Report of the Wellcome Research Laboratories, p. 148.
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THE OCCURRENCE OF A SPIROCHAETE IN THE GUT OF *GLOSSINA TACHINOIDES*

In the course of some experiments with trypanosomes a number of tsetse flies were dissected at Ikotobo in December, 1913, and examined for flagellates. In the lower half of the gut of one *G tachinoides*, a female, innumerable spirochaetes were found. The fly had been fed for some days on a clean guinea-pig, but although the blood of this animal was repeatedly examined, no spirochaetes were ever found in it.

In the fresh preparation the gut was seen to be packed with a writhing mass of spirochaetes. The organisms appeared to move with great activity. When stained, the spirochaetes were mostly found to be aggregated into clumps and tangles, in which it was impossible to follow the individual parasites, but numerous isolated specimens were also present, and a number of these was drawn and measured. Their lengths varied greatly, both long and short forms being found; the former measuring 15 μ to 35 μ, and the latter 7 μ to 10 μ. The spirals were ill-defined and variable, but the longer individuals generally showed five or six distinct undulations, and the shorter ones two or three. The extremities of the spirochaetes were blunt, and the parasites were extremely slender.

Novy and Knapp (1906)[9] have described a spirochaete occurring in the stomach of *G. palpalis*, and have named it *S. glossinae*. They describe it as occurring as single short forms measuring 8 μ in length, and double forms about 15 μ long. The ends of the spirochaete were indistinct and tapering. The short forms had four turns, but the authors noted that in some instances the spirals were drawn out into rather straight forms. The parasite found in *G. tachinoides* at Ikotobo would appear therefore to be distinct from this species.

Nothing is known as to the further history of this parasite. The guinea-pig on which the fly had fed repeatedly did not develop

spirochaetosis, and subsequent experiments, in which wild *G. tachinoides* were fed on fowls, were negative. The tsetse appeared to be unaffected by the infection, and had been kept in captivity for twenty-two days before being killed for examination.

REFERENCE

9. Novy, F. G., and Knapp, R. E. (1906). Journ. of Infect. Diseas., Vol. 3, p. 385.

EXPLANATION OF PLATES

PLATE XXIV

All the figures were drawn from preparations stained with Giemsa's solution. The blue coloration of the corpuscles in figs. 1 and 2 was due to restaining after the slides had been exposed to the vapour of creosote.

Figs. 1-29. Babesia of sheep and cattle, probably *Theileria parva*. × 2,000. Figs. 1 and 2 from a sheep at Lagos; figs. 3-6 from a sheep at Onitsha; fig. 7 from a dwarf cow at Ikotobo; figs. 8 and 9 from a Hausa cow at Calabar; and figs. 10-19 from a ram at Lagos. Figs. 20-29 from cultures of the blood of the ram at Lagos.

Figs. 30-37. *Babesia sp.* from a Hausa cow at Calabar. × 2,000.

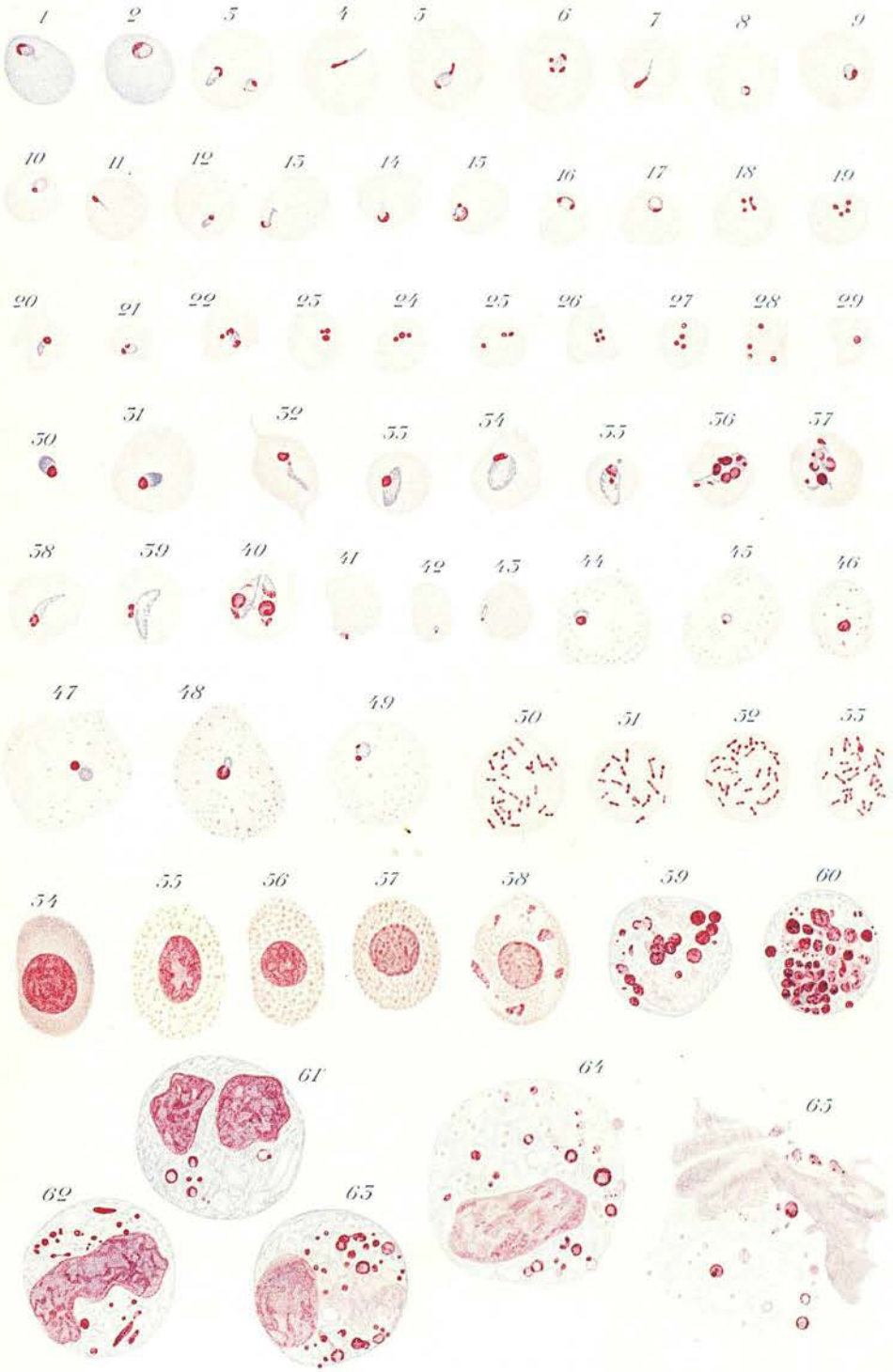
Figs. 38-40. Babesia from a dwarf cow at Ikotobo, probably *B. bigemina*. × 2,000.

Figs. 41-43. Babesia of guinea-pigs closely resembling *Paraplasma flavigenum*. × 1,000.

Figs. 44-49. Babesia-like parasite of guinea-pigs—*Paraplasma cobayae sp. nov.* × 2,000. Fig. 46 depicts one of the naked chromatin masses not infrequently seen which may, or may not be a stage in the development of the parasite.

Figs. 50-53. *Grahamella* from a brown rat at Ikotobo. × 2,000.

Figs. 54-65. Blood corpuscles from sick fowls at Eket and Ikotobo, × 2,000. Figs. 54-58, red blood corpuscles showing the stippling, and the atypical staining of the nuclei. Figs 59-64 leucocytes, fig. 59 from the spleen, fig. 60 from the liver, and figs. 61-64 from the blood, showing the characteristic granules and rings. Fig. 65 a leucocyte which has burst and liberated the granules.



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BLOOD PARASITES COLLECTED IN NIGERIA

PLATE XXV

All the figures were drawn from preparations stained with Giemsa's solution.

Fig. 1. Slender type of filarial embryo found in lizards (*Agama colonorum*). × 1,000.

Fig. 2. Filarial embryo found in toads (*Bufo regularis*). × 1,000.

Fig. 3. Stumpy type of filarial embryo found in lizards (*Agama colonorum*). × 1,000.

Fig. 4. Dumb-bell shaped body found associated with the slender type of filarial embryo (fig. 1) in lizards (*Agama colonorum*). × 1,000.

Figs. 5, 6, 7, and 9. Haemogregarine of toads (*Bufo regularis*). Figs. 5 and 9 large endoglobular forms. × 2,500. Figs. 6 and 7 merozoite-like and free forms. × 2,000.

Fig. 8. Haemocystidium, probably *Haemoproteus agamae*, of lizards (*Agama colonorum*). × 2,000.

Figs. 10 and 11. *Trypanosoma rotatorium* found in toads (*Bufo regularis*). × 1,500.

Fig. 12 *Trypanosoma sp.* found in a lizard (*Agama colonorum*). × 1,500.

Fig. 13. *Trypanosoma mega* found in toad (*Bufo regularis*). × 1,500.

Figs. 14 and 15. *Trypanosoma sp.* found in a toad (*Bufo regularis*). × 1,500.

Figs. 16-21. Haemogregarine found in lizards (*Agama colonorum*). × 1,000. Figs. 16-19 in red corpuscles, fig. 20 free, and fig. 21 in a leucocyte.

Figs. 22-35. Spirochaetes isolated in cultures of the blood of a guinea-pig. × 2,000. Fig. 22 small forms. Fig. 28 one of the very long forms. Figs. 29-35 forms showing the breaking up of the core.

M. Rhodes pinx.

BLOOD PARASITES COLLECTED IN NIGERIA

14

A NOTE ON

FIVE CASES OF POROCEPHALIASIS IN MAN FROM SOUTHERN NIGERIA

BY

J. W. SCOTT MACFIE, M.A. CANTAB., M.B., CH.B. EDIN.;

AND

J. E. L. JOHNSTON, M.B., B.S. LOND., D.T.M. & H. CANTAB.

(From the Medical Research Institute, Lagos.)

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A NOTE ON FIVE CASES OF POROCEPHALIASIS IN MAN FROM SOUTHERN NIGERIA.

Up to the time when Sambon published his important paper on Porocephaliasis in Man[1] apparently but few cases had been recorded. In a subsequent paper[2] the same author added to his list four other records. It has, however, been pointed out by Löhlein[3] that the infrequency of the occurrence of this condition which might be deduced from the small number of cases on record does not really exist. He records how Seiffert found porocephali in 7·8 per cent. of necropsies performed on coloured railway workers in the Cameroons, and how he himself, in a series of 118 post-mortem examinations on negroes, found 10 infected with these larvæ. Borden and Rodhain have recorded cases from the Congo also, so that it would appear that porocephaliasis is not very uncommon in the natives of West Africa.

The five cases briefly described below occurred in Southern Nigeria. The first occurred at Ibadan, a large native town situated about 120 miles north of Lagos, the second at Calabar, the third at Itu, and the fourth and fifth at Degama. We are indebted to Dr. J. D. Finlay for the interesting account of the first case, to Dr. J. Currie for the notes on the second, and to Dr. A. H. Wilson for the records of the remaining three.

Case 1.—The patient was a native man, aged about 24, who was brought to the Ibadan Hospital on July 31st, 1913. He died immediately after his admission, and in consequence the history of his case is somewhat

[1] Journal of Tropical Medicine and Hygiene, July 15th, 1910.
[2] Ibid., April 1st, 1913.
[3] Beiheft zu Archiv für Schiffs- und Tropen-Hygiene, 1912, vol. xvi., No. 9.

vague. He is said, however, to have been taken ill five days previously, and the symptoms observed were fever and vomiting. At the post-mortem examination, performed by Dr. Finlay, the body was found to be well nourished, rigor mortis was present, and the conjunctivæ were noticed to be deeply stained. All the organs and tissues were intensely jaundiced. Lungs: The right lung was normal; the left lung was contracted, and the pleural cavity was filled with venous blood and dark clot. Heart: The cardiac muscle was soft. No valvular lesion was present. Liver: The liver was greatly enlarged, bile-stained, and somewhat soft. On its surface, just below the peritoneal covering, were several porocephalus larvæ, and in the substance of the organ a few more were found. There were also some lying free on the under surface of the liver. The gall-bladder was filled with thick bile. On section the liver showed the condition known as "fatty nutmeg liver." Stomach: The stomach was contracted and thickened, and Dr. Finlay compared its appearance to that of an ox. Several round worms were present in the cavity. Duodenum: The duodenum was surrounded by a hard mass which blocked the common bile-duct. On microscopical examination this mass appeared to be of the nature of an adeno-carcinoma. Intestines: The small intestine appeared to be normal, but the ascending colon was adherent to the hard mass referred to above. Kidneys: The kidneys were enlarged and stained a dark green colour. The larvæ on examination proved to be those of porocephalus armillatus. Three individuals measured 18 mm., 16 mm., and 15 mm. in length, and comprised 21, 19, and 17 rings respectively. The rings in the middle of the body were 1 mm. apart, and stood out prominently. The breadth of all three was 3 mm. The cephalo-thorax appeared to be identical with that figured by Sambon for this species.

CASE 2.—The second case was that of an old Hausa woman who was found dead by the police at Calabar in September, 1913. Dr. Currie, who was called upon to make a necropsy, determined that death had been due to extensive and long-standing dysentery. In the course of his examination he found a single larva of porocephalus armillatus encysted in the edge of the liver. No more of the parasites were found in other situations.

CASE 3.—The patient, whose age was about 40, was a native of Southern Nigeria, and an inmate of Itu prison. He was a big, well-developed man. who did not appear to be seriously ill. Only a very indefinite account of his illness could be obtained. He first complained of being sick on August 5th, 1910, stating that he was constipated and had vague pains about the abdomen. At the same time he noticed that his legs began to swell. He was first

seen by Dr. Wilson on August 11th, and was found to have œdema of his legs, scrotum, and abdominal walls. The abdomen was distended, and contained free fluid. There was no enlargement of the liver and spleen, the urine was normal, and the heart and lungs appeared to be healthy. He was not very weak, and could get about fairly well. The patient was removed to Ikot Ekpene. On August 15th, when he was next seen, there was an increase of the dropsy ; dyspnœa was present, with a cough and frothy expectoration. On examination of the lungs moist crepitations were heard, and there was slight impairment of the resonance, especially on the right side. The patient died on August 18th. A post-mortem examination was made four hours after death. The body was well nourished. There was œdema of the legs and scrotum and slight general anasarca. On opening the abdomen a large amount of clear ascitic fluid escaped. There was no sign of peritonitis. The spleen was normal in size and appearance. The kidneys seemed to be a little congested. The liver was slightly enlarged, but in general appearance looked healthy. Scattered over its surface and in each lobe were what looked at first glance like cicatricial patches, but these proved on closer examination to be the encysted larvæ of porocephalus armillatus. Altogether there were 10 or 12 to be seen. They were all situated superficially, and were of a whitish colour. They were about half an inch long, closely coiled up, and surrounded by a thin membrane. Exactly similar larvæ were found in the lungs, but they were not so numerous as in the liver, and one of them lay an inch from the surface. The lungs were generally congested, and contained much frothy fluid. The bronchial tubes showed signs of congestion. There was no increase of fluid in the pleural cavities, a few slight adhesions were present, but there were no signs of recent pleurisy. The heart was normal. The pericardium contained an excess of fluid. The intestines were normal in appearance, and no larvæ were seen on their surfaces. The mesentery and the glands were not especially examined.

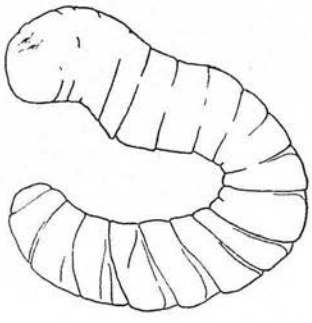
CASE 4.—The patient, a male native, aged about 50, was a prisoner from the Ahoada district of Southern Nigeria. In April, 1913, he was admitted to hospital at Degama for observation, but his symptoms appear to have been somewhat vague and indefinite, and in the same month he was transferred to Bonny. In August he was re-transferred to Degama. On re-admission it was noted that he was in "an emaciated and generally feeble condition," but no definite disease was diagnosed to account for his condition. His asthenia was progressive, and he could only walk feebly for a few yards at a time. He was discharged on Sept. 5th, but remained in the prison awaiting transport to his own country. About the same date it was observed that his abdomen was somewhat swollen, and on exa-

mination the presence of free fluid was detected. Two days later the feet and legs became œdematous, and asthenia became extreme. On the 11th dyspnœa and cough supervened, and the lungs were found to be congested. There was no fever and the urine was normal. Two days later the patient died. At the post-mortem examination, which was made by Dr. Wilson on the same day, the body was found to be emaciated and the legs slightly œdematous. Thorax: The heart appeared to be normal. There were a few old adhesions on both sides in the pleural cavities. Both lungs were much congested, and on the surfaces ecchymoses were found. On section a large amount of blood and frothy fluid escaped, the condition being one of general congestion and œdema. The lungs were searched for larvæ, but none was found. Abdomen: On opening the abdomen a large amount of limpid fluid escaped. There was no peritonitis. The liver was normal in colour and appeared to be below the usual size, and was tough in consistency. On its surface was a number of cicatricial patches. On further examination a nodule about ⅓ inch in diameter was found on the surface of the right lobe. This proved to be an encysted larva enclosed in a very tough and fibrous capsule. On thoroughly searching the liver four similar encysted larvæ were found. The mesentery was searched for larvæ, but with negative result. The spleen, kidneys, pancreas, and stomach and intestines were all normal.

CASE 5.—The patient, a Kroo boy, aged about 17 years, was admitted to Degama Hospital on July 7th with ulceration of the cornea of the right eye. He made no further complaint, and the examination of his physical condition was negative. He was, however, reported to have been losing strength recently and to have been performing his work in a languid fashion. Whilst in hospital his condition was one of gradual and progressive asthenia, and he died on July 14th. Two weeks before his death his gait became staggering, his knee reflex was lost, and he complained of some vague pains about the legs; but with the exception of rapid action of his heart no local signs or symptoms could be detected, and no evidence of cardiac incompetency was found. At the post-mortem examination the body was found to be fairly well nourished and presented no abnormal appearances. Thorax: The pericardium was adherent to the heart, the right ventricle thin and dilated, the left ventricle normal, and the valves normal. The lungs were very small but appeared to be healthy; no porocephalus larvæ were found although careful search was made for them. Old pleuritic adhesions were present on both sides. Abdomen: The abdominal cavity contained a little free peritoneal fluid, but there were no signs of peritonitis. The liver was slightly enlarged, there was general hepatitis, and the organ presented a nutmeg appearance. On the

surface two patches with a superficial resemblance to cicatrices were found, which when cut into were seen to be encysted larvæ of porocephalus. On making successive sections through the liver about ten of these larvæ were found. None was more than a half to three-quarters of an inch beneath the surface. One larva was found in the mesentery. The kidneys, pancreas, stomach, and intestines were all normal.

The parasites from the last case had unfortunately been considerably damaged in dissection, and only one complete individual could be pieced together. This specimen (see figure) consisted of 20 rings, and



Porocephalus from Case 5, magnified. Actual length, 17 mm.

measured 17 mm. in length and 3 mm. in breadth. An unopened cyst from the same case measured 9 mm. by 6 mm. by 3 mm. The larvæ differed somewhat from those of the other cases. They were smaller, and the rings, which were broad and band-like, were set more closely together. The heads also appeared to be more bulbous. Such differences in appearance may have been due to the larvæ having been in an immature condition.

Very various opinions are held with regard to the effects of porocephalus larvæ on their human host. Löhlein seems to believe that they are harmless, but Chalmers states: "The presence of the parasite in the human body sets up inflammation of the lungs and peritoneum, but it does not appear to irritate the alimentary canal or liver."

In the first two cases recorded above sufficient evidence of disease was detected at the necropsies to account for death without attributing any action to the presence of the larvæ. In the second case only a single porocephalus larva was found in the body, but there was evidence of severe and chronic dysentery, and in the first death would appear to have been due to the presence of a tumour surrounding the duodenum and blocking the common bile duct, and the few symptoms noted, and the pathological appearances observed, were probably due to this cause. The possibility that irritation caused by wandering larvæ might have played some part in the production of the tumour should not, however, be forgotten, although no evidence of any such predisposing cause was detected at the necropsy. The three cases recorded by Dr. Wilson (Nos. 3, 4, and 5), on the other hand, suggest that the presence of porocephalus larvæ may have serious pathological significance. No definite cause of death was discovered at the post-mortem examinations, and Dr. Wilson himself considered that the fatal termination in each case had at any rate been accelerated by the presence of the larvæ. These three cases presented a peculiar clinical picture, and the pathological conditions found after death were somewhat similar. The chief symptoms were progressive weakness, œdema, and dyspnœa, without any very definite signs to account for the physical conditions. The patient in Case 5 developed some interesting nervous symptoms shortly before death. A larva was found in the mesentery, a fact which suggests the possibility that wandering larvæ may have been responsible for some of the symptoms. It is interesting to note that Waldow found small cysts containing calcified porocephali in the liver, omentum, mesentery, and pleura of a native who died in the Cameroons with symptoms of meningitis.

A NOTE ON THE ACTION OF COMMON SALT ON THE LARVAE OF STEGOMYIA FASCIATA.

BY

J. W. SCOTT MACFIE, M.A., M.B., Ch.B.

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1914.

A NOTE ON THE ACTION OF COMMON SALT ON THE LARVAE OF STEGOMYIA FASCIATA.

By J. W. Scott Macfie, M.A., M.B., Ch.B.,

West African Medical Staff.

(Plate XXXII.)

It has been pointed out by Graham* that measures designed to bring about the destruction of mosquito larvae may be divided into (*a*) measures intended to destroy the larvae, and (*b*) measures intended to destroy their food supply. He points out that very little attention has been paid to the second method, which entails an exact knowledge of the freshwater algae. Graham found that the suspended matter in water in which the larvae of *Pyretophorus costalis* were breeding could be precipitated by the addition of 3 per cent. of common salt, and that then the larvae became cannibalistic and destroyed one another. He considered that this phenomenon was due to the larvae being deprived of their natural food, the algae, which had been destroyed by the addition of salt. "In lesser concentration," he concluded, "salt appears to inhibit the growth of very young larvae, probably by diminishing the supply of food, but the development of fully-grown larvae appears to be hastened in a hypertonic medium, and they pass into and through the pupal stage with unusual rapidity."

The following experiments were undertaken with a view to determining to what extent the action of salt on mosquito larvae was due to the destruction of the natural food supply, and to what extent to the hypertonic nature of the solution. The larvae employed were those of *Stegomyia fasciata*. This species was selected both because its breeding places would be most easily treated with salt, should this substance prove of value as a larvicide, and on account of its importance in a country in which yellow fever is endemic.

Salinity of the Medium in which the Larvae were found.

At Lagos the larvae of *Stegomyia fasciata* are commonly found in water-pots and domestic utensils in the compounds of the native quarters of the town. A number of samples of water were obtained from this source, and an analysis was made of the amount of salt present in each. The percentage was found to vary considerably, ranging from 0·005 % to 0·019 % NaCl, and the average of six determinations was 0·012 % NaCl.

This figure was found to be considerably below that for the water of the Lagos lagoon at the same season (September). The salinity of the water flowing beneath the Iddo bridge, to the North of Lagos, was found to be 0·026 % NaCl; whilst that at the Magazine bridge, considerably nearer the sea, was 0·112 % NaCl.

* Bull. Ent. Res. Vol. i, p. 51–52, 1910.

It may therefore be assumed as probable that the larvae of *S. fasciata* thrive best in a medium whose salinity is about 0·012 per cent. NaCl. In order to determine whether the higher percentage of salt present in the lagoon would prove injurious to them, three samples of water containing larvae from domestic utensils was selected, and salt was added to them until the salinity of each was 0·026, the same percentage as was found to be present in the lagoon water at the Iddo bridge. No perceptible action was observed on the larvae, which remained healthy and active during the succeeding four days. The percentage of salt in two of the samples was then raised to 0·112, as in the lagoon water under Magazine bridge. The larvae seemed to be affected somewhat by this degree of salinity, and tended to remain for long periods at the bottom of the jars. By the seventh day all those in the one sample were dead, and only one remained alive in the other. The last larva died on the eighth day.

Experiments with Solutions of 0·5 per cent. NaCl and upwards.

The foregoing preliminary experiments suggested that it might be of some interest to determine the effects of various strengths of salt solutions on the larvae of *S. fasciata*. In order to exclude the factor of precipitation, the experiments were in the first instance carried out in clean water.

For this purpose ten mature larvae were placed in each of six jars containing respectively 0·5 %, 1 %, 2 %, 3 %, 4 %, and 5 % NaCl solutions. The results of the experiments are shown in detail below :—

TABLE I.

NaCl.	0·5 %	1 %	2 %	3 %	4 %	5 %
Oct. 12, 11 a.m.	10 L.	10 L.	10 L.	10 L.	10 L.	10 L.
,, 1 p.m.	9 L., 1 P.	10 L.	9 L., 1 P.	10 L.	1 L., 9 dead L.	All dead.
,, 3 p.m.	9 L., 1 P.	10 L.	9 L., 1 P.	7 L., 3 dead L.	All dead.	
,, 5 p.m.	9 L., 1 P.	10 L.	9 L., 1 P.	6 L., 1 dead L.		
,, 11 p.m.	9 L., 1 P.	10 L.	9 L., 1 P.	All dead.		
Oct. 13.	7 L., 3 P.	7 L., 3 P.	8 L., 1 P., 1 dead L. Three larvae almost inert.			
Oct. 14 ...	6 L., 4 P.	7 L., 2 P., 1 M.	4 L., 1 P., 3 dead L.			
Oct. 15 ...	5 L., 2 P., 3 M.	6 L., 3 M.	All dead.			
Oct. 16 ...	5 L., 2 P.	6 L.				
Oct. 17 ...	5 L., 2 M.	4 L., 2 dead L				
Oct. 18 ...	5 L.	4 L.				
Oct. 19 ...	5 L.	4 L.				
Oct. 20 ...	5 L.	3 L., 1 dead L.				
Oct. 22 ...	5 L.	1 L., 2 dead L.				

L = Larva, P = pupa, M = adult mosquito.

The effects of the different solutions on the larvae varied directly with their strengths. In 5 % NaCl all the larvae had died within two hours, in 4 % within four hours, and in 3 % within twelve hours. In 2 % NaCl solution all had died within three days ; in 1 % during the ten days for which the experiment was continued, five larvae died, four pupated and hatched, and one remained alive at

the conclusion of the observations. The lowest concentration, 0·5 % NaCl, seemed to have but little effect; five larvae pupated and hatched, and five remained alive at the end of the experiment; none died.

The rapidity with which the larvae were killed by the stronger solutions seems to prove that, at any rate as low as 2 or 3 % NaCl, the action on the mature larvae of *S. fasciata* is an osmotic one, and is not dependent on the destruction of algae, nor on the deprivation of the insects of their natural food supply.

The action of the salt solution is not, however, appreciably altered by the presence of débris, etc., as is proved by the following experiment. A large number of larvae and pupae in their natural medium were placed in a jar, and enough salt added to bring up the strength of the solution to 2 %. Six hours later many of the larvae were dead, and twelve hours after this all had perished. A number of the pupae hatched during the first two days, but on the third day all those that remained were dead.

Experiments with more dilute solutions of salt.

Although solutions of salt of 2 % and upwards appeared to produce an inimical effect on the larvae of *S. fasciata* by direct osmotic action, more dilute solutions in clear water had a much less pronounced effect. It was considered possible, however, that the latter solutions might cause a precipitation of the organic constituents of the natural fluid in which the mosquito larvae were found, and might thus kill, or at any rate inhibit the growth of, the larvae by depriving them of food as was suggested by Graham.

Mature larvae were therefore distributed into glass jars, and the salinity of the natural medium, which contained 0·012 % NaCl, was increased by the addition of salt to 0·10 %, 0·15 %, 0·20 %, 0·25 %, and 0·30 % respectively. Larvae were also introduced into a solution of 0·32 % NaCl in pure water for comparison. The results of these experiments are shown below.

TABLE II.

NaCl.	0·10 %	0·15 %	0·20 %	0·25 %	0·30 %	0·32 % in pure water.
Oct. 11.	20 L.	20 L.	10 L.	20 L.	20 L.	10 L.
Oct. 12.	17 L., 3 P.	13 L., 2 P., 5 dead L.	5 L., 1 P., 4 dead L.	9 L., 4 P., 7 died L.	9 L., 1 P., 10 dead L.	7 L., 1 P., 2 dead L.
Oct. 13.	11 L., 9 P.	9 L., 4 P., 2 dead L.	2 L., 3 P., 1 dead L.	4 L., 6 P., 3 dead L.	8 L., 2 P.	5 L., 3 P.
Oct. 14.	8 L., 12 P.	3 L., 9 P., 1 dead P.	5 P.	1 L., 7 P., 2 M.	2 L., 7 P., 1 M.	5 L., 3 P.
Oct. 15.	2 L., 9 P., 8 M., 1 dead P.	1 L., 8 P., 3 M.	2 P., 3 M.	4 P., 3 M., 1 dead L.	7 P., 1 M., 1 dead L.	3 M., 5 dead L.
Oct. 16.	1 L., 8 P., 2 M.	3 P., 3 M., 3 dead P.	2 M.	2 P., 2 M.	2 P., 5 M.	
Oct. 17.	3 P., 4 M., 2 dead P.	3 M.		1 M., 1 dead P.	1 L., M., 1 dead P.	
Oct. 18.	1 P., 2 M.					
Oct. 19.	1 M.					

L = Larva; P = pupa; M = mosquito.

It will be observed that during the first 24 hours the effect of the saline solutions in the natural medium, estimated by the death of the larvae, was proportional to the strengths of the solutions used. In the experiment with clear water the action was not marked until three days later. It is possible that the injurious effects in the former cases may have been due to the clogging of the tracheae of the larvae by precipitates. The deaths during the first 24 hours cannot at any rate be attributed to starvation.

All the larvae in these experiments had either died, or pupated and hatched, by the 9th day. The number that died was roughly in proportion to the strength of the saline solution. Thus in the 0·10 % solution none died as larvae, but in the 0·30 % eleven of the original twenty died in this condition.

NaCl.	0·10 %	0·15 %	0·25 %	0·30 %
Hatched	17	9	8	8
Died as pupae	3	4	1	1
Died as larvae	0	7	11	11

Very young larvae appeared to be more adaptable than mature larvae to a change in the concentration of the medium. Solutions containing 0·10 %, 0·20 %, and 0·30 % NaCl did not appear to have any appreciable effect on them.

Experiments with Alum Solutions.

If, as has been suggested, the inimical action of common salt on mosquito larvae is due to the precipitation of the minute algae on which they feed, it might be supposed that alum would have a more pronounced effect owing to its well-known clarifying action on impure waters. It was thought possible also that the gelatinous precipitate might interfere with the respiratory processes of the larvae, for it had been observed that in a pool containing innumerable larvae great numbers had died when some toad spawn was deposited in it. The spawn floated on the surface of the water and spread out like a veil, the eggs being connected by fine threads of mucus in which the mosquito larvae had become entangled and had died.

Three experiments were carried out in which potash alum was added to turbid water containing larvae so as to bring the strengths of the solutions up to 0·05 %, 0·10 %, and 0·20 % respectively. Ten mature larvae were used in each experiment.

The addition of the alum produced a copious precipitate which slowly settled to the bottom of the jars, but there was no immediate effect on the vitality or activity of the larvae. In the jar containing 0·05 % solution five larvae pupated and hatched, one larva died on the 7th day, and four that had pupated died on the third and fourth days. The last pupa hatched on the 12th day. Alum in this concentration appeared to have little or no direct effect. In the jar containing 0·10 % solution only one mosquito hatched, and the other nine larvae died between the third and the seventh days. In the third jar containing 0·20 % solution seven larvae died between the fourth and the seventh days, and three

that had pupated died on the third, fourth, and sixth days respectively. It was evident therefore that alum in these concentrations had no peculiar action on the larvae.

In more dilute solutions in clean water the results were inconclusive. Of ten mature larvae in a 0·01 % solution three pupated and hatched, and seven died as larvae; in a 0·02 % solution exactly the same results occurred; and in a 0·03 % solution two pupated and hatched and eight died as larvae. All had either hatched or died by the eighth, ninth, and tenth day respectively.

The action of a medium containing salt and alum in equal parts was tried in another series of experiments. The results, however, were similar to those obtained with a corresponding concentration of common salt alone.

Summary of Results.

The foregoing experiments would seem to prove that, in solutions of 2 % and upwards, the action of common salt on the larvae of *Stegomyia fasciata* is due to the hypertonicity of the solution. In more dilute solutions the destruction of the natural food supply of the larvae may have some influence. Alum, however, which clarifies water more efficiently than common salt, has no peculiar action on the larvae.

In Lagos the larvae of *Stegomyia fasciata* are found most abundantly in water contained in domestic utensils, and in the large pots in which the natives store up water for drinking and cooking. It would be of great advantage if common salt could be used as a larvicide in these cases; for not only would the water not be rendered unfit for use in cooking, as it is by the application of kerosene, but also evaporation would tend to increase the strength of the larvicide, and repeated applications would be unnecessary. It would also be possible to keep stores of water in the compounds without danger. From the experiments described, it would appear that sufficient salt would have to be added to each vessel to bring the concentration of the solution up to 2 % NaCl in order to ensure the destruction of the larvae.

The Survival of Mosquito Larvae in Temporary Pools.

It often happens that small pools of water, such as those illustrated by the accompanying photographs (Pl. XXXII), collect beneath the taps of tanks and at the open ends of drains, and into these mosquito larvae are apt to be swept. Such pools soon dry up, the water seldom lying in them for more than an hour or two, and they may thus escape the attention of the Sanitary Inspectors. The soil round them is, however, permanently wet; and if, as is usually the case, the pools are renewed once a day or more frequently by the tanks or drains being used, it is quite possible that mosquito larvae might develop in them. In some cases the finer soil washes away leaving a mass of irregularly-shaped stones in the interstices between which water may lie concealed all day long. Such pools may occasionally account for the presence of mosquitos in a house near which no breeding place can be located.

With a view to determining to what extent mosquito larvae were capable of surviving intermittent desiccation of this kind, the conditions obtaining under a

tank like that shown in Pl. XXXII. fig. 2 were reproduced in the laboratory by means of tins in the bottom of which small holes were pierced. The tins were half filled with soil and small stones, and an inch of water was added on the top. The drainage was arranged so that the surface of the soil became exposed after about one hour. The artificial pools thus formed were filled up every morning, and allowed to drain away as described. Thus a free surface of water was present for about one hour each day only. The soil, however, remained moist throughout the day.

Into such artificial pools pupae, mature larvae, and young larvae of *Stegomyia fasciata* were introduced. In the case of pupae the majority always hatched in the course of three or four days. In one experiment started with ten pupae, four had hatched within the first 24 hours, three more during the next 24 hours, and two more during the third day; only one pupa perished. Mature larvae were found to be much less resistant to desiccation. They not infrequently pupated, but in our experiments none of the pupae hatched. Young larvae also survived only a short time, and did not undergo their normal development. Some of them however, remained alive until the sixth day; and it is possible that had the pools been renewed more often, or allowed to persist a little longer, as no doubt they often do under natural conditions, the larvae might have matured, pupated and even hatched out into mosquitos.

The chief danger of such occasional pools therefore is that pupae, washed into them from other pools or from the gutters of bungalows, may complete their development in them. But as even immature larvae may also live for a number of days in such situations, it would perhaps be a wise precaution to treat these places with larvicides.

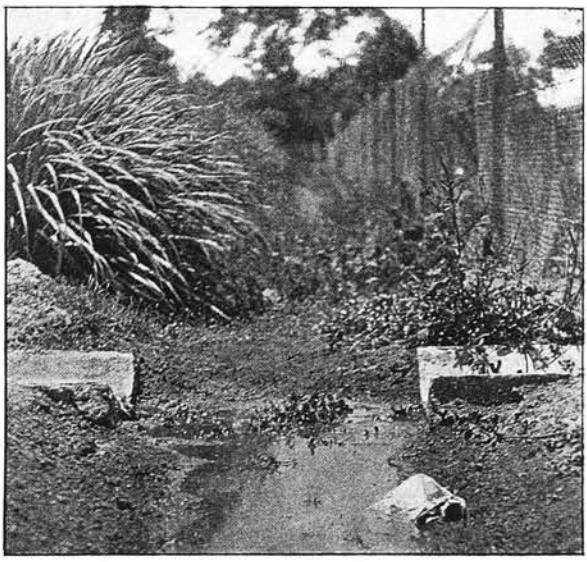

Fig. 1. Temporary pool at the open ends of a drain.

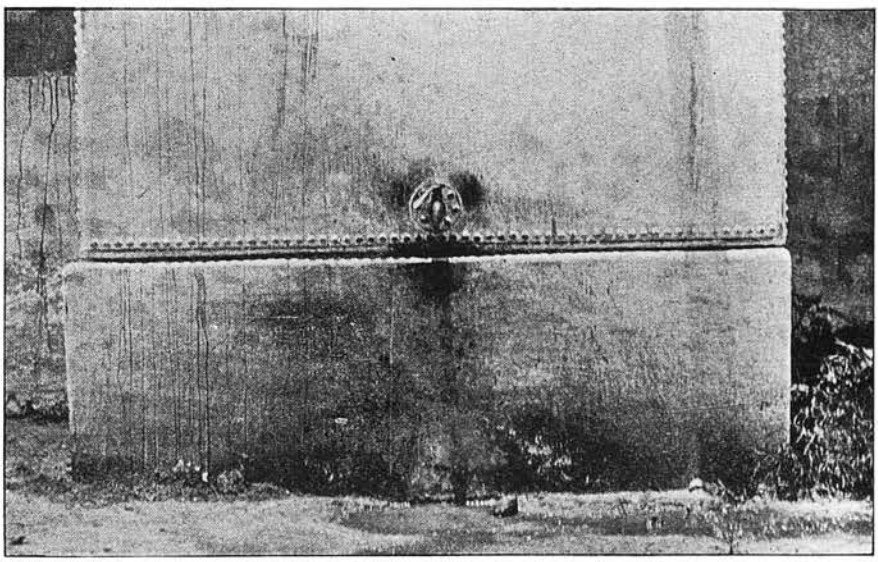

Fig. 2. Water-tank, showing the pools that collect under the tap.

BREEDING PLACES OF STEGOMYIA FASCIATA, LAGOS, S. NIGERIA.

ON A NEW AFRICAN SPECIES OF COCCIDAE.

By J. W. Scott Macfie, M.A., B.Sc.,
West African Medical Staff.

Ceronema africana, sp. n.

Female (fig. 1).—Ovisac formed of thickly felted creamy-white to buff-white secretion ; dorsum highly convex longitudinally and slightly so transversely, with a series of 11 longitudinal and crenulated keels or ridges, of which the lateral ones are very broad and flange-like and arise from points on the body of the female considerably nearer the middle line than any of the others ; sides vertical, with two or three curved or concentric keels similar to those on the dorsum, but

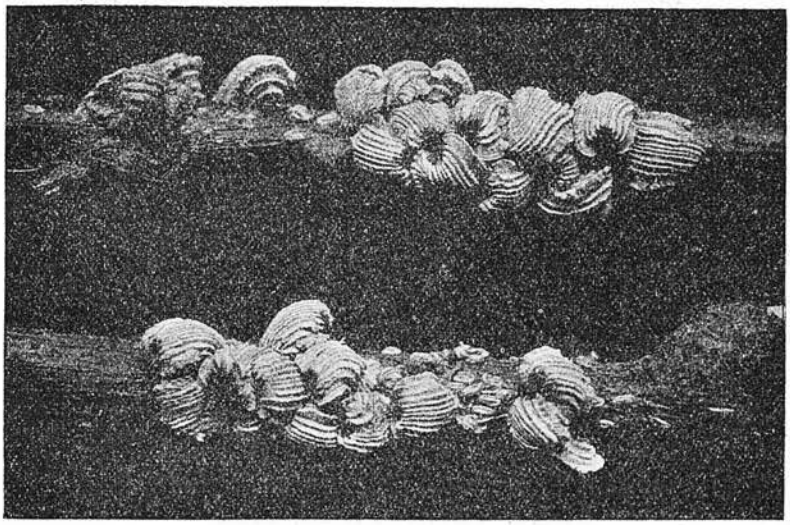

Fig. 1.—*Ceronema africana*, Macfie ; females with ovisacs *in situ* ; very slightly enlarged.

there are also small transverse intervening lines which give the sides of the ovisac a distinctly conchoidal appearance ; interior of ovisac smooth. *Length*, 6·50 to 8·50 mm. ; height, 6 to 7·50 mm. ; width, 6 to 7·25 mm. Old adult female tilted as in Pulvinaria so that the front, which is distinctly produced, is the only portion attached to the food-plant. In some instances the position of the body is almost vertical, and in one example the anal portion projects actually slightly in front of the cephalic margin. Dorsum with a median elongated patch of opaque glassy secretion, the rest nude and dark brown in colour.

Female after maceration in potash—broadly ovate, and usually distinctly produced anteriorly. *Derm* covered irregularly with more or less circular cells (fig. 2 A) with thick walls ; in the centre of each cell a circular aperture and a

minute pore with a narrow canal communicating with it. *Abdomen* covered ventrally with small circular spinnerets especially at the posterior extremity and along the periphery. The main spinning glands (figs. 2 B *a*, 2 C *a*), corresponding to the ridges of the ovisac, placed singly round the margin of the body, just dorsal to the marginal spines. Anal lobes (fig. 2 F) longer than in *C. koebeli*, Green,* each furnished with a variable number of spinose hairs at either pole; ano-genital ring with ten stout hairs. Margin with a close series of short

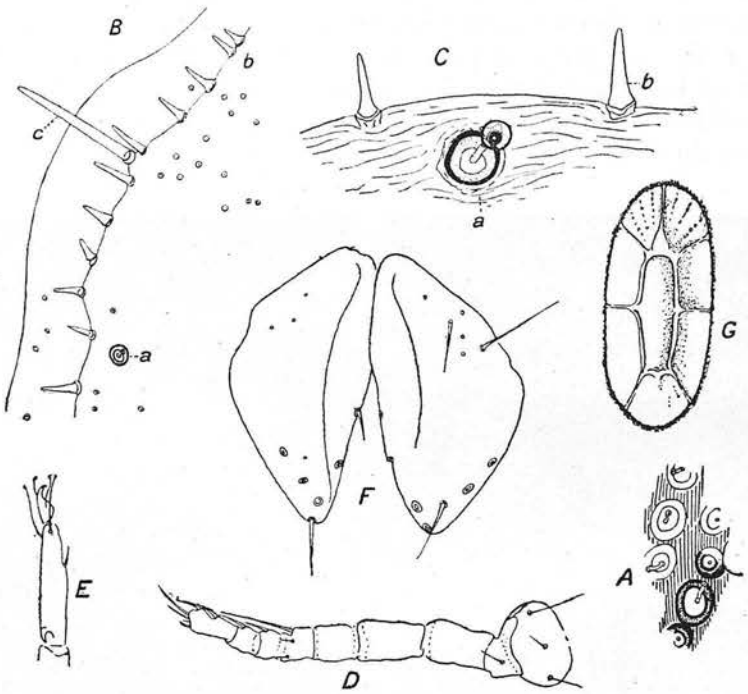

Fig. 2. *Ceronema africana*, Macfie.

Female.—(A) derm-cells; (B *a*) large spinning gland; (B *b*) marginal spines; (B *c*) stigmal spine; (C) spinning gland and marginal spines more highly magnified; (D) antenna; (E) tarsus; (F) anal lobes.

Male.—(G) male puparium.

pointed spines (figs. 2 B *b*, 2 C *b*); a single larger spine (fig. 2 B *c*) marking the stigmatic area. *Antenna* (fig. 2 D) nine-jointed; third and fourth joints considerably longer than the others, the third, fourth, and fifth apparently without any hairs; one particularly long hair is attached to the sixth joint. *Legs* relatively small but well developed; foot (fig. 2 E) with four digitules, dilated distally, claw strongly hooked at the tip and with a minute tubercle on the concave border near the extremity. *Length*, 6·2 to 8 mm.; width 4·5 to 5 mm.

* Coccidae of Ceylon, p. 256, pl. xcvi, figs. 1–16 (1909).

LARVA (taken from the ovisac of the female)—elliptical. Antennae (fig. 3 A) six-jointed, the third joint being the longest; two especially long hairs on the third and sixth joints. Stigmatic area (fig. 3 B) marked by one long and two short spines, which are slightly spatulate at their tips, and a row of three or four daisy-shaped spinnerets (fig. 3 B *a*) leading inwards towards the stigma. Margin with a series of small pointed spines. Legs well developed. Caudal setae (fig. 3 C *a*) very long, in some cases measuring as much as two-thirds of the length of the body; at the base of each seta a group of four spines. Ano-genital ring (fig. 3 C) with six stout hairs.

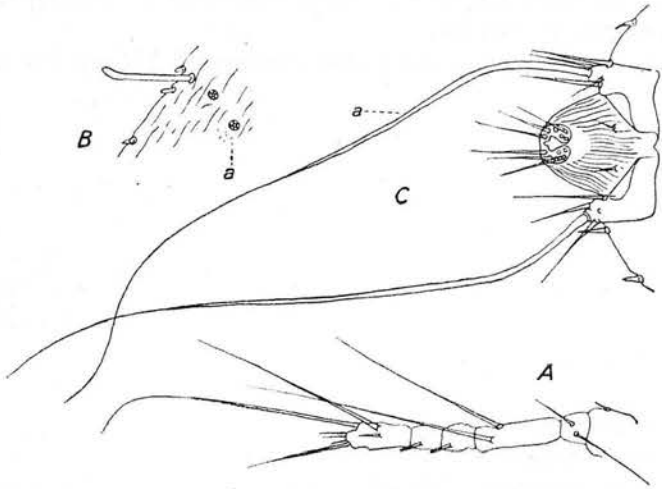

Fig. 3. *Ceronema africana*, Macfie.

Larva.—(A) antenna; (B) stigmal spines; (B *a*) spinnerets; (C) anal segment; (C *a*) caudal setae.

MALE.—*Puparium* (fig. 2 G) glassy opaque white, elongated, with parallel sides; form not differing materially from that of the genus *Lecanium*; margin with an imperfect and irregular fringe of waxy appendages. *Length* 2·5 mm., breadth 1·5 mm.

Adult male unknown.

NORTHERN NIGERIA: Shonga, Ilorin Province, iii. 1912.

Food-plant.—"Pride of Barbados" (*Caesalpinia pulcherrima*, Sw.), a prickly shrub growing to a height of 5 to 10 ft., which, on account of its showy red or yellow flowers, is a favourite in the gardens and compounds of Nigeria.

In the form of the ovisac of the female this species bears a striking resemblance to *Ceronema koebeli*, Green (*l.c.*), but morphologically the latter is very clearly distinct. The distinctive characters in *C. africana* are that the antennae are decidedly stouter, the digitules of the claw are less dilated, the central stigmal spine is considerably longer, and there is a total absence of short spines leading up to the stigmata. Furthermore the derm cells are oval in shape and not "irregularly polygonal" as in *C. koebeli*.

This is apparently the first recorded occurrence of this genus in Africa.

I have to thank Professor Newstead for the greatest kindness and assistance in working out the characters of this species.

Dactylopius (Pseudococcus) virgatus var. **madagascariensis,** Newst., Liverpool University Quarterly Journal, iii, no. 6.

I have to record here this species as occurring plentifully on the young shoots of a tree called by the natives "chedia" (*Ficus sp.*) at Ilorin, Northern Nigeria, in May 1912. This insect has been recorded elsewhere as feeding upon cactus, cocoanut palm, cotton, violets, etc.

This species is also apparently new to the continental African fauna.

TABANIDAE ATTRACTED BY SCALE-INSECTS.

Dr. J. W. Scott Macfie, Medical Officer at Ilorin, Northern Nigeria, has made the interesting observation that a tree attacked by a Coccid of the genus *Ceroplastes* served as a strong attraction to several species of TABANIDAE, including both males and females. He writes :—

"I am sending a small lot of flies representing exactly a week's collecting from one small 'Chedia' tree in my compound, which I have just discovered to be an abundant source of biting flies. The 'Chedia' is very common all over the country; it exudes quantities of milky latex on the slightest injury, and bears small reddish-yellow berries, but I do not at present know its scientific name. In my compound I have several young trees of this species, but the one on which all these flies were taken differs from the others in being heavily infested with a species of scale-insect. It is quite a small tree, not more than 14 feet high, and the largest of its branches is only 2½ inches in diameter. The surface of all the branches is more or less covered with old scales, and on the young growing shoots there are fresh scales that attract swarms of ants, which march in columns up the tree-trunk all day long. The flies, however, are never seen on these young shoots; they always appear suddenly and silently on the large branches over which they crawl, slowly moving their fore feet to and fro laterally, as though they were sweeping invisible atoms into the middle line under their probosces, which every now and then they bend down, appearing to be feeding. So engrossed are they that it is almost possible to catch them by hand.

"I see, in the Sleeping Sickness Bulletin (No. 36), in the review of a paper by Prof. Hine on North American TABANIDAE, that 'many species of the family TABANIDAE have been observed feeding on the excretions of insects' such as 'aphides, scale-insects and Hemiptera.' One male (*Tabanus gratus*), which I dissected, had its stomach full of small yellow bodies which did not stain with Giemsa's solution. The flies are only to be found on the tree in the day-time, and especially when the sun is shining; I have never found them on the branches at night.

"It is noteworthy that my pony, tethered not 20 yards away, was quite unmolested by flies at the very times when I was catching considerable numbers on this 'Chedia' tree. Indeed, until I observed this source I was able to get but few specimens of biting flies here (Ilorin). It is, perhaps, a danger to have such a tree in the vicinity of a house or stable, on account of its influence in attracting blood-sucking insects, although the majority of the flies may prefer the diet found on the tree to the meal of blood afforded by the horse."

The specimens sent by Dr. Macfie comprised sixty TABANIDAE, referable to the following six species :—

	♂	♀
Tabanus pluto, Walk.	1	—
„ *biguttatus croceus*, Wied. ...	3	3
„ *taeniola*, P. de B.	9	11
„ *gratus*, Lw.	15	6
„ *laverani*, Surc.	3	4
Haematopota sp. nov.	3	2

The males of *T. biguttatus croceus* and *T. laverani* had not been previously recorded.

Prof. R. Newstead, F.R.S., has kindly identified the scale-insect which attracted the flies as *Ceroplastes egbarum*, Ckll. (*africanus*, Green). The species was originally described from Abeokuta, in Southern Nigeria, but appears to be widely distributed in Africa. Prof. Newstead remarks that "many of the LECANIINAE, of which *Ceroplastes* is a member, secrete a kind of 'honey-dew' that is attractive to insects, especially Hymenoptera; and in Great Britain all the species of the genus *Vespa* are particularly fond of the secretion."

BULL. ENT. RESEARCH, III, pt. 2 (1912).